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LXXXIV. THE INFLUENCE OF SATURATION DEFICIENCY AND OF TEMPERATURE ON THE COURSE OF EPIDEMIC PLAGUE.

By RALPH ST JOHN BROOKS, M.D., D.P.H.

(With 18 Charts.)

From the Lister Institute of Preventive Medicine.

The prompt decline of plague epidemics in Northern India with the onset of the hot weather led many observers to the conclusion that the establishment of a critical temperature was the most important factor in checking the course of the infection. The facts concerning the seasonal prevalence of plague in six selected places in India were examined by the Plague Commission (1) and the conclusion was arrived at "that a plague epidemic is checked when the mean daily temperature passes above 80° F. and especially when it reaches to 85° F., or 90° F." The fact that at high temperatures the plague bacillus disappears from the stomach of the flea more rapidly than at lower temperatures has been suggested as a possible explanation.

The question was subsequently investigated by the Commission (2) and (3) with special reference to Belgaum and Poona, localities in which plague epidemics tended to decline under presumably favourable conditions of temperature, and it was shown that variations in atmospheric humidity had an important relation to the seasonal prevalence of the disease and that variations in the percentage humidity of the atmosphere were associated with variations in the average number of fleas found per rat at different times of the year.

The relative humidity figures of a locality do not, however, give precise information as to the drying capacity of the air at the temperature in question, and it was thought that if from the percentage humidity and temperature figures, "saturation deficiency" figures were derived, these might afford more useful information for the analysis of plague data in the light of atmospheric conditions. By "saturation deficiency" is meant the difference between the actual tension of aqueous vapour present in the atmosphere at the temperature in question and the tension of aqueous vapour that would be present in a saturated atmosphere at the same temperature.

The importance of expressing the hygrometric condition of the atmosphere in terms of saturation deficiency will be readily apparent if it be remembered that dry air at 50° F. has actually a smaller saturation deficiency, and hence a smaller drying capacity, than 80 per cent. saturated air at 100° F.; the saturation deficiency in the former case being ·360 of an inch, in the latter case ·384 of an inch.

The Commission has collected and correlated data for plague deaths and the temperature and percentage saturation of the atmosphere for a number of localities in India during the past few years. From these figures I have calculated the saturation deficiencies and replotted them in relation to plague deaths. The data for a number of localities not studied by the Commission have also been treated in the same way. By expressing the facts in this way the possible influence of variations in dryness and variations in mean temperature on the incidence and course of epidemic plague in India can be separately studied and how far the influence of the former may be exercised independently of the factor of temperature.

It has been pointed out by Greenwood (5) that the factors that cut a plague epidemic short are more determinate than those responsible for its commencement. The decline of plague in a locality doubtless may depend on circumstances other than climatic, for in isolated outbreaks in the same district the epidemic subsides in one village at the same time that it is developing in another. Nevertheless, the seasonal incidence of the disease in India is one of the characteristics of plague epidemiology.

From the examination of a large number of charts plotted to show (*a*) the mean monthly temperature, (*b*) the mean monthly saturation deficiency, and (*c*) the percentage above and below the mean of the monthly plague deaths for the locality, or the average monthly deaths for five plague years, it is quite apparent that in a considerable number of cases as soon as the mean temperature rises above 80° F., the epidemic rapidly declines. In most localities, however, a rise of temperature is associated with a corresponding increase in saturation deficiency, so that in these cases it is impossible to assess the importance of temperature and dryness. It is only by the study of the climatic conditions in a number of regions that the differential effect becomes manifest.

The cutting short of the epidemic at a temperature at or above 80° F. is, in all those cases I have been able to investigate, associated with a saturation deficiency of ·30 of an inch or upwards, and this association is so constant that it seems quite reasonable to suppose that there is a real and critical relation between the two figures in so far as either of them have effect in checking epidemic plague. The chart of Bombay (see Chart I) gives a good example of this association, the epidemic curve falling rapidly as soon as the temperature passes 80° F.; the saturation deficiency remaining at or about the ·30 of an inch mark for some time. The mean temperature remains at about 80° F. until the month of November, but from the month of June onwards the saturation deficiency is not unfavourable to plague, and indeed autumn recrudescences of plague in Bombay are by no means uncommon. The charts of Lahore (see Chart II) and Lucknow (Chart III) are very similar and may be cited as other examples of the epidemic being checked by the rising of the mean temperature to over 80° F., though in these cases the saturation deficiency is considerably higher than ·30 of an inch at that temperature. At the time the saturation deficiency reaches ·30 of an inch in these localities the mean temperature is under 70° F., and at this temperature such a degree of deficiency does not appear to be sufficient to influence the course of the epidemic. The charts of Ballia (see Chart IV) and Darbhanga (see Chart V) afford additional examples of

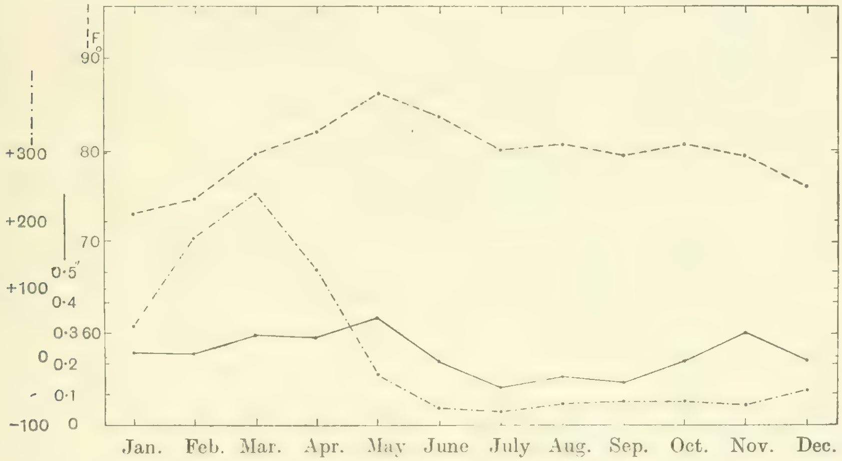

Chart I. Bombay.

------ Temperature.

Saturation deficiency.

-·-·-·- Plague deaths. (Average above and below the mean for years 1896–1904.)

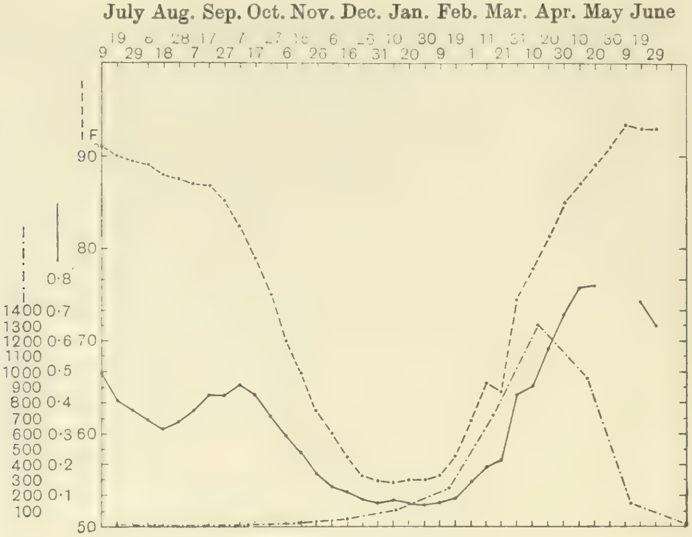

Chart II. Lahore.

------ Temperature.

—— Saturation deficiency.

-·-·-·- Plague deaths. (5 years' average.)

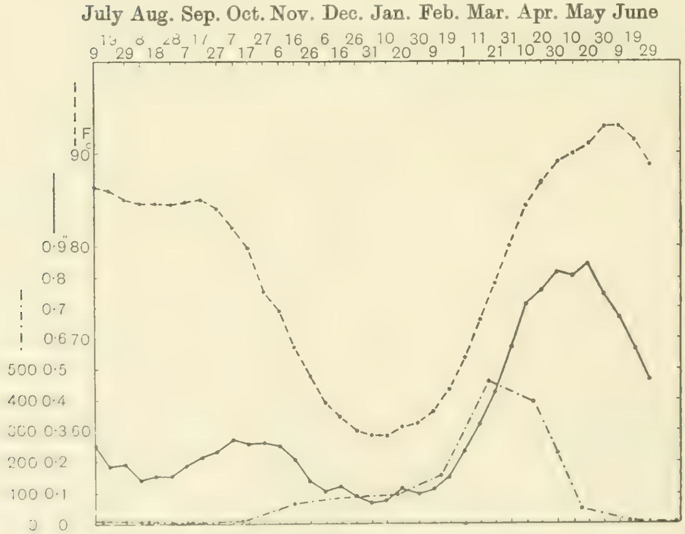

Chart III. Lucknow.

------ Temperature.

—— Saturation deficiency.

-·-·-·- Plague deaths. (5 years' average.)

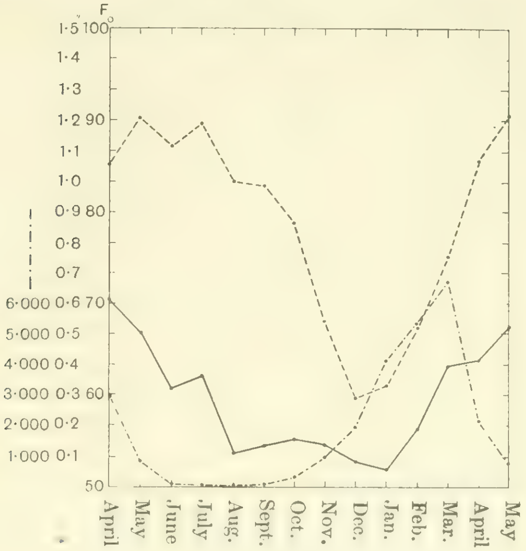

Chart IV. Ballia. April 1911—May 1912.

------ Temperature.
—— Saturation deficiency.
-•-·-·- Plague deaths.

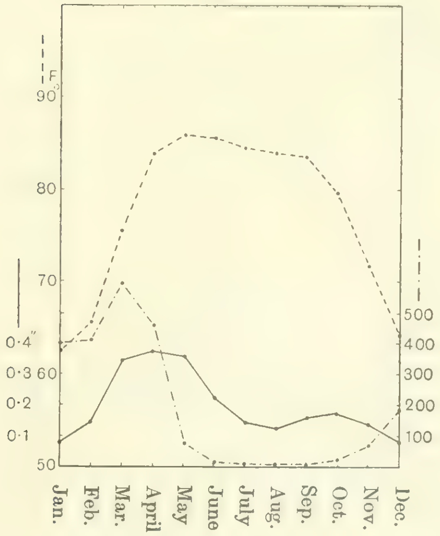

Chart V. Darbhanga.

------ Temperature.
—— Saturation deficiency.
-·-·-·- Plague deaths. (5 years' average.)

a falling epidemic associated with a rising temperature and saturation deficiency.

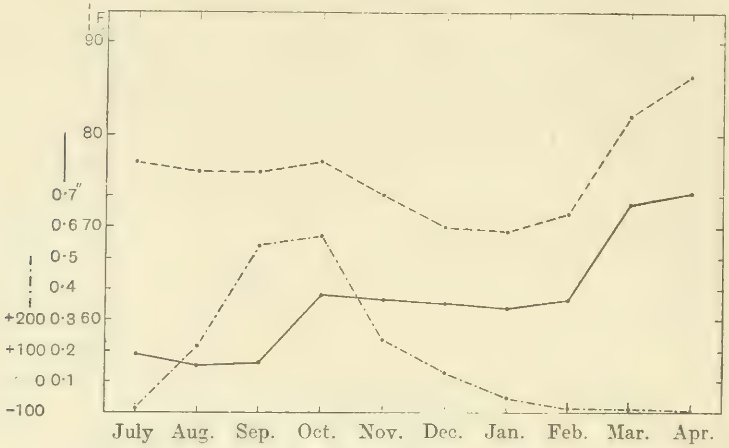

Chart VI. Poona. Early plague years 1897–8, 1900–1 and 1906–7.

------ Temperature.
———— Saturation deficiency.
-·-·-· Plague cases. (Average above and below mean.)

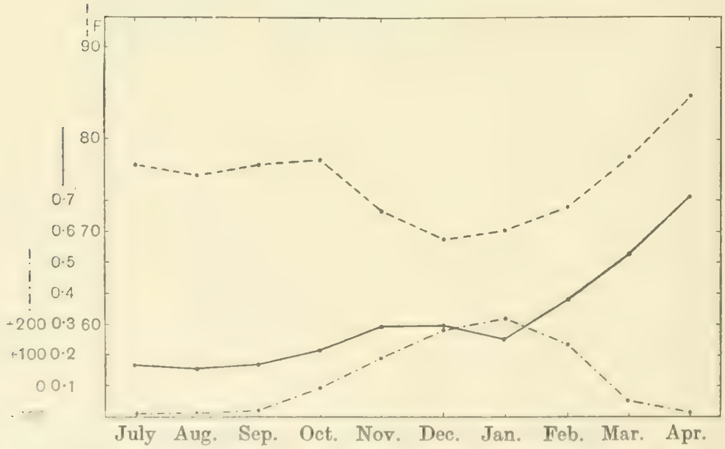

Chart VII. Late plague years 1901–2, 1902–3, 1903–4 and 1904–5.

------ Temperature.
———— Saturation deficiency.
-·-·-· Plague deaths. (Average above and below mean.)

If, however, an appropriate degree of saturation deficiency be present, the epidemic may decline long before the mean temperature reaches 80° F. and may indeed be practically over before that event occurs.

In this connection an examination of the epidemics in Poona is of peculiar interest, as in this locality the usual epidemics may be divided into two groups, (*a*) a group in which the outbreak occurs between August and November, and (*b*) a group in which the epidemic season is roughly November to February. The two accompanying charts (see Charts VI and VII) are plotted out from the average figures for three years of early epidemics (1897–1898, 1900–1901, 1906–1907) and for four years of late epidemics (1901–1902, 1902–1903, 1903–1904, 1904–1905) respectively. It will be seen that in both charts the mean temperature does not reach 80° F. until the epidemic season is over, so that, as already pointed out by the Commission, the cutting short of the Poona epidemics cannot be due to the effect of temperature *per se*.

It will however be observed that in the group of early epidemics the saturation deficiency rises to nearly ·4 of an inch in the month of October, coincident with the falling of the epidemic, and is associated with a mean temperature of 77° F. A study of the group in which the epidemic is of late occurrence, reveals quite a different state of affairs, for here the saturation deficiency does not rise above ·30 of an inch until the month of February, and this increase of the saturation deficiency is again correlated with the decline of the epidemics. The two charts reveal practically the same mean temperature conditions month for month throughout the year, so that were it not for the fact that there is no apparent explanation why the epidemics in 1901–2, 1902–3, and 1904–5 should have been delayed in *onset*, the conclusion that saturation deficiency is in each case responsible for the decline of the epidemic would be irresistible.

The chart of Nagpur (Chart VIII) during the epidemic of 1903–1904 presents another example suggesting the effect of a rapid rise of saturation deficiency abruptly terminating a plague epidemic at a mean temperature considerably below 80° F. The epidemic commenced under favourable conditions of temperature and saturation deficiency in September, rose to its height in December–January and then rapidly fell with the increasing saturation deficiency. In the month of February the saturation deficiency had risen to ·44 of an inch, while the temperature was but 74° F. The epidemic was at that time rapidly declining, and by the time the temperature reached 80° F. the saturation deficiency was nearing ·60 of an inch and the epidemic was practically at an end. When the deficiency was at ·30 of an inch the epidemic was still raging, the temperature being about 70° F., whence it appears that a saturation deficiency that is instrumental in checking plague at 80° F. is not sufficient to have the same effect at a lower range of temperature (70° F.).

The chart of Jhansi in the Bundelkhand district of the United Provinces (Chart IX) shows a similar condition of affairs. The curves are plotted to show the course of the epidemic of 1911–1912, with the temperatures and saturation deficiencies for the epidemic period.

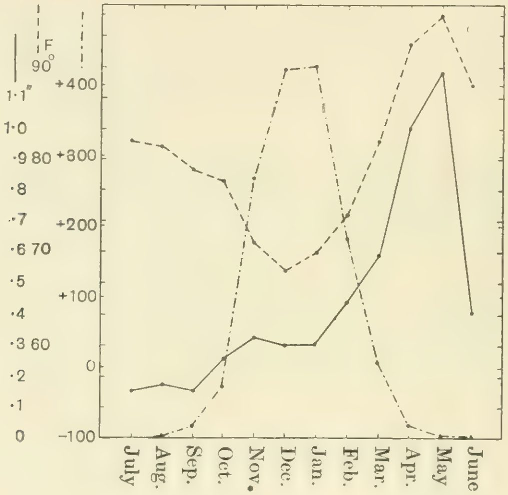

Chart VIII. Nagpur. Epidemic of 1903–4.

------ Temperature.
——— Saturation deficiency.
-·-·-·- Plague deaths. (Average above and below mean.)

During most of the year the high temperature and high saturation deficiency were quite unfavourable for the occurrence of epidemic plague. The first plague cases occurred in the month of November, when conditions became favourable, and the height of the epidemic was reached in February, when the temperature was about 70° F. and the saturation deficiency about ·30 of an inch. The epidemic declined very rapidly, *pari passu* with a sudden rise in the saturation deficiency, and by the middle of March was well under way; the temperature at that time being only 76° F. but associated with a saturation deficiency of ·67 of an inch.

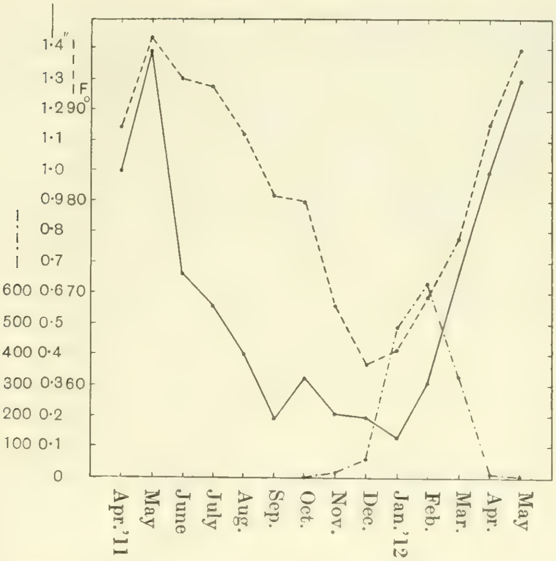

Chart IX. Jhansi. April 1911—May 1912.

------ Temperature.
———— Saturation deficiency
-·-·-·- Plague deaths.

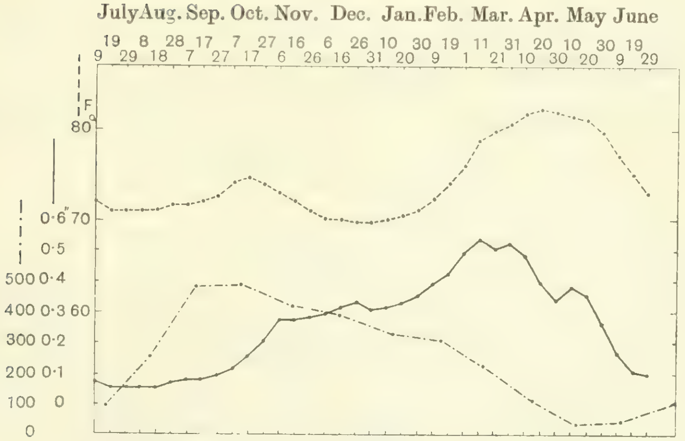

Chart X. Belgaum.

------ Temperature.
———— Saturation deficiency.
-·-·-·- Plague deaths. (Average of 5 years.)

The chart of Belgaum (Chart X) again shows the epidemic declining under favourable conditions of temperature but unfavourable conditions of saturation deficiency for such temperature. By the time the temperature has reached 80° F., the saturation deficiency has reached ·50 of an inch and the epidemic has practically spent itself. Belgaum was at no time absolutely free from plague, but during the months of April and May, when the conditions are the most adverse, the mortality is comparatively slight.

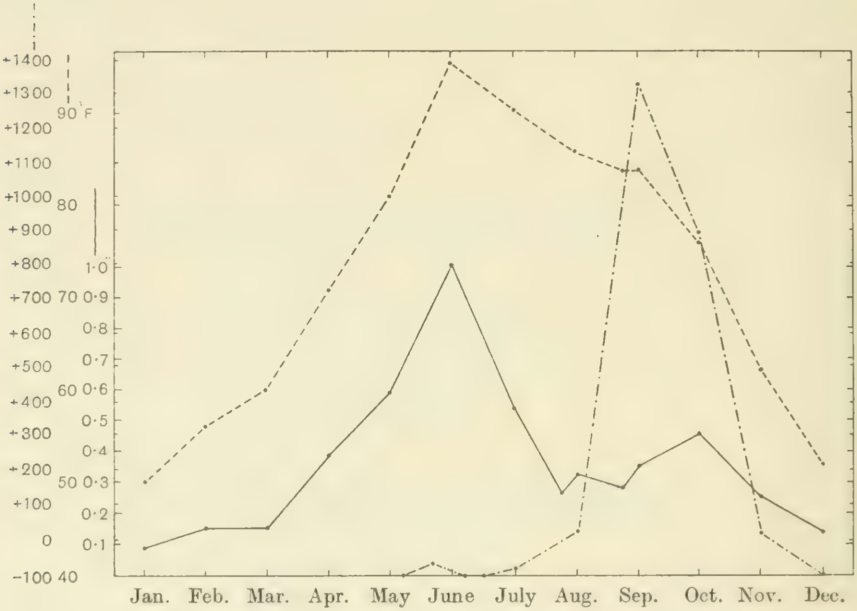

Chart XI. Rawalpindi. First epidemic, 1903.

------ **Temperature.**

———— **Saturation deficiency.**

-·-·-·- **Plague deaths. (Average above and below mean.)**

The influence of the saturation deficiency on the commencement of the epidemic.

If an examination be made of all the charts previously referred to, it will be seen that the epidemic season for plague started, in all cases, when the mean temperature was well below 80° F. As previously pointed out, in India this temperature is usually associated with a saturation deficiency of ·30 of an inch or upwards, and in such conditions it does not appear that plague can assume epidemic proportions. When, however, a temperature of 80° F. and upwards is associated with a low saturation deficiency, plague may become epidemic in spite of a high mean temperature. The chart of Rawalpindi (Chart XI), showing the first plague epidemic there, which occurred in the year 1903, reveals this interesting state of affairs. The disease, which was apparently slumbering during the hot and dry weather, suddenly assumed epidemic proportions in August-September 1903. Coincident with the rise of the epidemic the saturation deficiency fell in the early days of August to ·26 of an inch, having somewhat abruptly fallen from so high a deficiency as 1·07 of an inch since the middle of June. The temperature meanwhile had been steadily falling from 92° F., which it had attained in the month of June, but was still at the 84° F. mark when the epidemic was at its height in September. The epidemic then rapidly declined, as the saturation deficiency rose to ·45 of an inch in the month of October, the mean temperature *falling* meanwhile to 76° F.

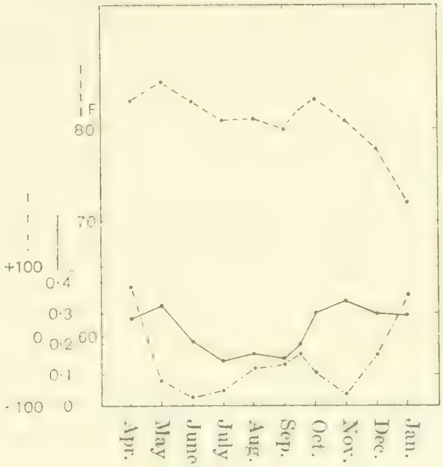

Chart XII. Autumn recrudescence of plague in Bombay, Aug.–Sept. 1898.

------ Temperature.
—— —— Saturation deficiency.
-·-·-·- Plague deaths. (Average above and below mean.)

An examination of plague data in connection with the port of Bombay reveals the fact that there the epidemic season reaches its height in March or April. It has, however, been observed that in some years slight recrudescences occur in the months of August and

September. So far as I am aware no satisfactory explanation has yet been given with regard to these "autumn epidemics."

During the period in question the temperature is usually above 80° F. and to that extent would not unreasonably be considered as unfavourable to recrudescent plague. An examination of the chart (see Chart XII) of the autumn recrudescence of 1898 shows clearly that during the whole period under consideration the mean temperature never fell below 80° F., but that the rise in the plague curve was associated with a very low degree of saturation deficiency. As the saturation deficiency approached ·30 of an inch with the mean temperature over 83° F., the number of plague cases rapidly fell. In November the temperature dropped to below 80° F. with the saturation deficiency remaining about ·30 of an inch and plague again resumed epidemic proportions. The chart of Salem (see Chart XIII) reveals the same state of affairs, though to a less marked degree than is the case of Rawalpindi. Plague occurs here at all months in the year but reaches its lowest level during the comparatively hot and dry months of April, May and June. In the month of July the saturation deficiency

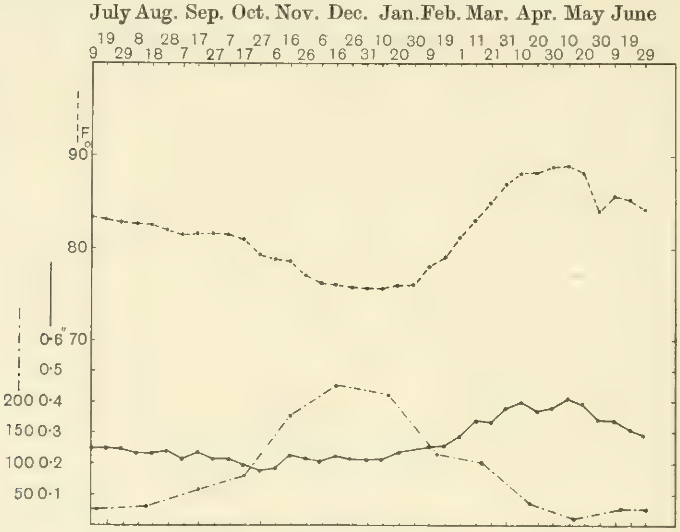

Chart XIII. Salem.

------ Temperature.
———— Saturation deficiency.
-·-·-·- Plague deaths. (Average of 5 years.)

falls below ·30 of an inch, while the temperature is still above 80° F. This state of affairs continues till about the middle of October, the number of plague cases rising steadily the while. The plague incidence is considerably increased as soon as the temperature falls below 80° F.; the saturation deficiency remaining at about ·20 of an inch.

The climatic conditions met with in Rangoon, Burma, are quite different from those which obtain in India proper, and are of peculiar interest in an investigation of the present nature. It will be observed (see Chart XIV) that for nine months in the year the mean temperature is above 80° F., falling at no time, during the remaining three months, below 76° F. During the month of April the mean temperature rises to over 87° F., at a time, be it observed, when the plague mortality is at its highest. The saturation deficiency throughout the year is, however, exceptionally low, varying between the limits ·06 and ·26 of an inch. In Rangoon, in spite of the high mean temperature throughout the year, the saturation deficiency is exceptionally low, and judging from experience elsewhere it cannot be said that there is any season of the year at which the climatic conditions are unfavourable for the development of plague. It is true that the number of cases of plague during the months October to December are comparatively

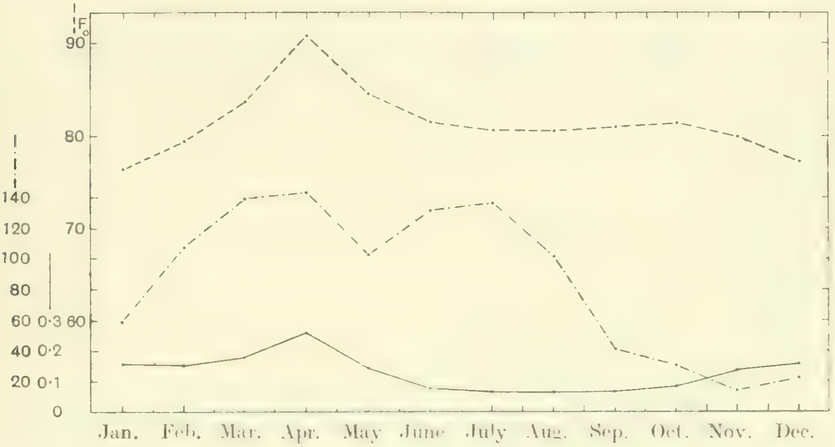

Chart XIV. Rangoon.

------ Temperature.
———— Saturation deficiency.
-·-·-·- Plague deaths. (Average of 5 years.)

few, but as the climatic conditions at this time of the year are by no means antagonistic to the maintenance of epidemic plague, an explanation of the reduction in the case incidence and mortality from this disease during these months must be due to other causes.

An examination of charts derived from particulars given by Van Loghem and Swellengrebel (6) with regard to plague in Java, Dutch East Indies, is interesting and instructive. The first chart (see Chart XV) shows the incidence of plague in the Melang Department of Java, from its introduction in April 1911 to October 1912. Although the time of the east monsoon is spoken of as the "dry period" and the time of the west monsoon as the "wet period," there is extraordinarily little variation in the temperature and saturation deficiency throughout the year—the temperature keeping within the limits 74–77·5° F. and the saturation deficiency within the limits ·13–·27 of an inch. The climate is not at any time unfavourable to the spread of plague, and it is not surprising to find that there is no marked epidemic season and that plague occurs with apparent indifference at all times of the year. The sudden rise and fall of the disease after its introduction cannot be explained on the grounds of adverse conditions of temperature or saturation deficiency and may be due to some hitherto unrecognised cause. Plague continued in epidemic form in the Melang Department

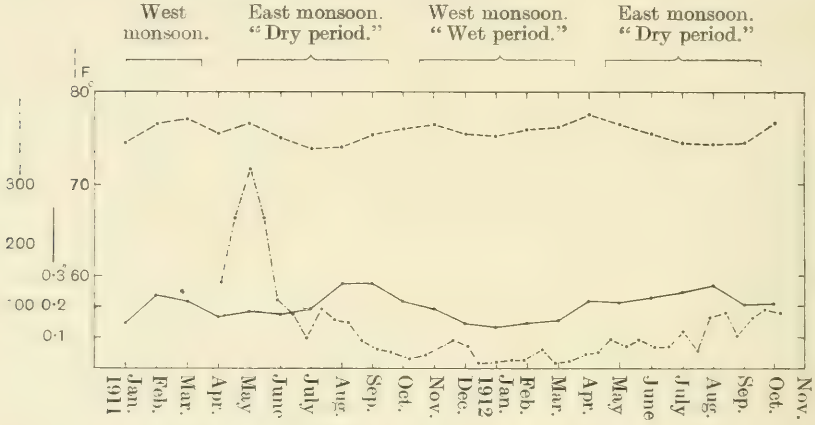

Chart XV. Java: Melang Department. First two years of plague, 1911 and 1912.

- - - - - - Temperature.
———— Saturation deficiency.
-·-·-·- Plague deaths.

throughout the year 1913 (see Chart XVI), the number of cases increasing from the month of August onwards.

The climate of Passuruan is said to resemble that of Surabaya and Maduin (Java). It will be observed by a reference to the chart (see Chart XVII) that, on the whole, it is somewhat drier and hotter than Melang and that towards the end of the dry season the mean temperature exceeds 80° F. with a saturation deficiency of ·35 of an inch, climatic conditions unfavourable for plague in India. A small number of plague cases occur at these places throughout the year and there does not appear to be any obvious epidemic season.

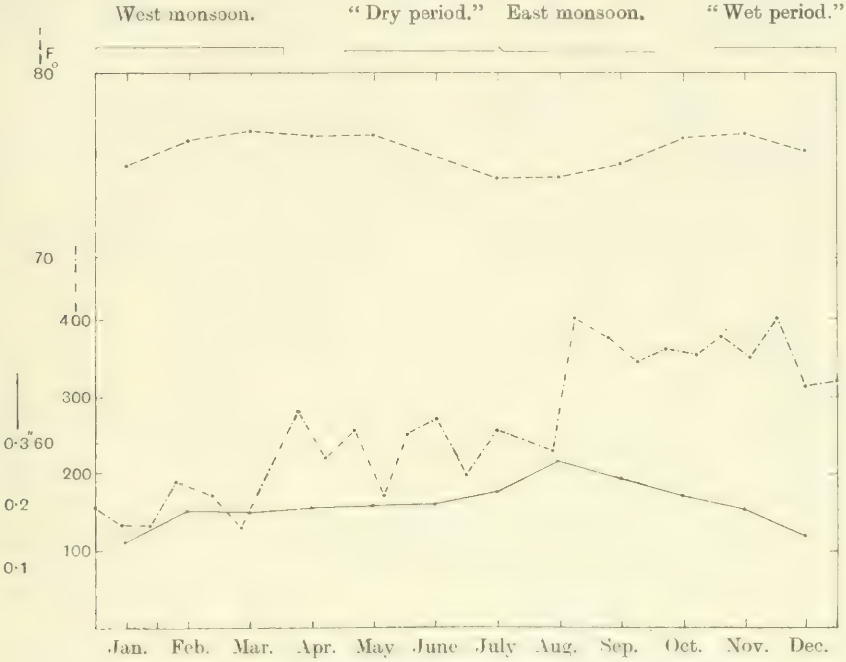

Chart XVI. Java: Melang Department. The plague epidemic of 1913.

------ Temperature. (Average of 1911 and 1912.)
——— Saturation deficiency. (Average of 1911 and 1912.)
—·—·—· Plague deaths.

The climate of Mauritius is similar to that of Java except that the temperature range is considerably greater, approaching 80° F. in the months of December and January (see Chart XVIII) and falling below 70° F. during the months June, July and August. The saturation deficiency is confined between the limits ·13 and ·27 of an inch. The chart shows the course of the first epidemic, plague being introduced into Mauritius from Madagascar in December 1898. The data given in the chart are abstracted from particulars given in the Report by the Acting Director of the Medical and Health Department of Mauritius (7). The disease did not get much of a foothold in the island during the warm weather, though, as we know from experience elsewhere, the climatic conditions were at no time unfavourable to the spread of plague. With the setting in of the cooler weather in June a rapid increase in the number of cases took place and the epidemic in the Port-of-entry, Port Louis, where the disease was first introduced, reached its height in August–September 1899.

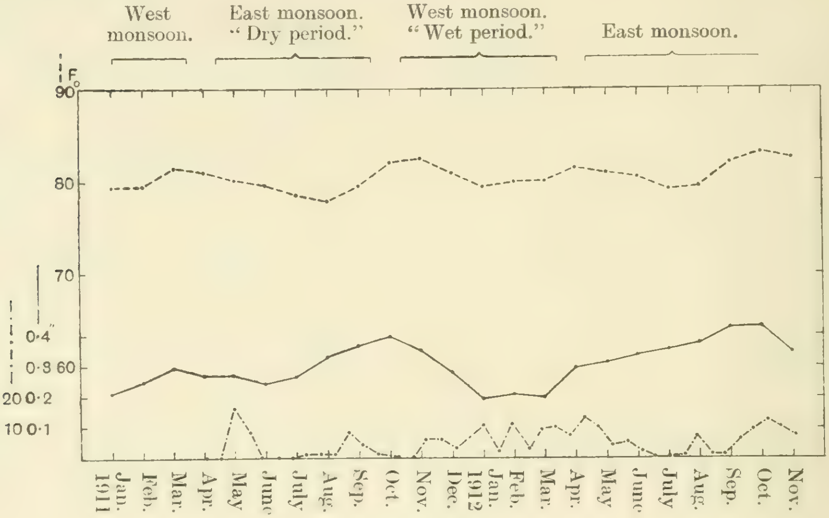

Chart XVII. Java: Surabaya and Maduin.

------ Temperature.

—— Saturation deficiency.

-·-·-·- Plague deaths.

From Port Louis other parts of the island in turn became infected, and subsidiary epidemics took place, which ran their own course, uninfluenced apparently by climatic conditions. The little epidemic at Pamplemousses, for example, reached its height in October and the outbreak at Plaines Wilhems was still increasing at the end of the year. These circumstances suggest that in places where the combined effects of temperature and saturation deficiency are at no time unfavourable to the occurrence and spread of plague, other factors come into play in determining the local epidemic.

It would thus appear that while the combined effects of temperature and saturation deficiency have in the majority of cases an influence on the incidence and course of plague epidemics, yet, under certain conditions, such epidemics come to an end at a time when the climatic conditions are presumably favourable for a continuance of the disease.

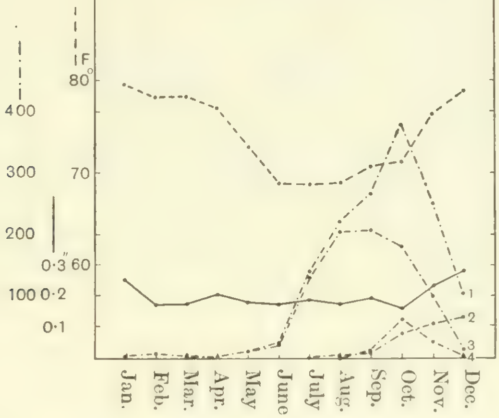

Chart XVIII. Mauritius. The first epidemic, 1899.

------ Temperature.
———— Saturation deficiency.
-·-·-·- Plague cases. 1, Whole Island; 2, Plaines Wilhems;
3, Port Louis; 4, Pamplemousses.

In these cases other factors must come into play, and attention is directed to the work of the Commission in connection with the seasonal breeding of rats, the decrease in the numbers of rats during epidemic periods and the accompanying increase in the proportion of immune to susceptible rats. The adverse influence of high temperature and saturation deficiency may be explained by their effect on the duration of life of the rat flea, *Xenopsylla cheopis*, when separated from its host.

When the mean temperature rises above 80° F. and when such rise is accompanied by an increase of the saturation deficiency to above

·30 of an inch, plague cannot maintain itself in epidemic form, though a high temperature *per se* may not bring about the termination of a plague epidemic. Many examples can be adduced of plague epidemics coming to an end when the temperature remains well below 80° F. and in such cases the determining factor appears to be the rising of the saturation deficiency to over ·30 of an inch.

Plague epidemics do not, as a rule, arise when the mean temperature is above 80° F. for the reason that at such temperatures it is quite exceptional to find a sufficiently low saturation deficiency. When, however, a high temperature occurs with a low saturation deficiency, plague epidemics do arise and maintain themselves. In Rangoon, where the temperature, although above 80° F. for over nine months in every year, is associated with an exceptionally low saturation deficiency, plague occurs at all seasons of the year. The autumnal recrudescences of plague in Bombay are found associated with a mean temperature of over 80° F. but with a low saturation deficiency of less than ·20 of an inch.

Generally speaking, it may be said that there is a critical saturation deficiency for each range of temperature. At 80° F. this critical saturation deficiency appears to be of the order of ·30 of an inch. At lower ranges of temperature a higher degree of deficiency is needed to suppress the epidemic, while at higher temperatures a somewhat lower deficiency will suffice.

Summary.

1. Plague does not maintain itself in epidemic form when the temperature rises above 80° F. accompanied by a saturation deficiency of over ·30 of an inch.

2. Plague epidemics are rapidly brought to an end in the presence of a high saturation deficiency even when the mean temperature throughout and after the termination of the epidemic has been considerably below 80° F.

3. Plague epidemics may commence and increase in intensity when the mean temperature is well above 80° F., provided that the saturation deficiency is below ·30 of an inch.

4. In some districts in India and in certain tropical islands (*e.g.* Java, Mauritius) where the climatic conditions are at all times of the year favourable to the incidence and spread of plague, the disease may occur indifferently at all seasons.

I desire here to express my thanks to Dr C. J. Martin, Director of the Lister Institute, at whose suggestion the above investigation was undertaken, for his kindly help and criticism during the progress of the work in question.

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THE INFLUENCE OF THE AGE OF THE PARENT AT BIRTH OF CHILD ON EYE-COLOUR, STATURE AND INTELLIGENCE.

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We have already considered, in previous papers, the influence of parental age, on birth, marriage, sex, susceptibility, and reaction to bacterial invasion, evidence having been produced which lends support to the belief that this factor does modify the organism in its behaviour with respect to the attributes considered. It is therefore reasonable to extend the enquiry, and examine whether these variations are accompanied by modifications in structural attributes. In the present paper the characters studied are, eye-colour, stature and intelligence.

Eye-colour was selected on account of its interest and comparative ease of observation: stature as a measure of growth: and intelligence, judged by scholastic standard, as a prime factor in the success of man in the eternal conflict between man and his animate or inanimate competitors.

Eye-colour.

Any general consideration of the enormous literature treating this subject is unnecessary: the work has already been done in great detail by Pearson, Nettleship and Usher, in their monograph on Albinism. All that is needful in the present instance is a reference to such works as have a direct bearing on the points discussed.

Before considering the actual data, some comment must be made on the tacit assumption that eye-colour is a fixed character, apparently not modified during the life of the individual. It should be remembered that all are born with blue eyes of varying shades, and that a proportion change to brown, grey, etc. The method by means of which this change is achieved is somewhat doubtful, and may be explained in two ways:

(*a*) by the deposit of a new layer of pigment in front of the choroidal layer, or (*b*) by the pigment already present undergoing a physical change, that is, the intracellular granules change from a molecular to a granular state. There is no histological evidence in favour of the former supposition, nor does direct observation of the iris reveal appearances which would lend credence to the belief that an anterior layer of pigment was being deposited.

The second hypothesis is in accord with the physical appearance of many substances when in different states. It is easily conceived that with such a pigment, placed in a semitranslucent or whitish stroma, with a slight tinge of red dependent on the capillary circulation, any known eye-colour could be obtained.

The rate at which this change takes place in childhood varies, and there is some evidence that even in adults the same tendency is observed.

The cause is doubtful, though the fact that the central areas of the iris are darker than the peripheral portions, does suggest that intensity of illumination is a factor in its production.

Pearson has already shown that even between the 5th and 20th years, there is a tendency for the eyes to darken in colour.

He gives the following values:

Virchow's data. Age and Eye-colour $C_r = \cdot 027$
Pletgour's ,, ,, ,, $C_r = \cdot 451$
Urchida ,, ,, ,, $C_r = \cdot 096$

The values seem to vary according to the number of years considered; he suggests that a selective death rate may explain the result.

The material here dealt with was collected in Middlesbrough from various sources and refers to females only. The standard selected was the number of blue-eyed persons in groups of different ages. They were reached in the following ways.

Infants through the Notification of Births Act.

School children through the Education Act (1908) and

Adults through the attendance of parents consequent on the above.

The groups, though not true random samples, are not selected with respect to the character under investigation, and hence should be suitable for analysis.

The results were as follows:

TABLE I.

Females only.

Age	No. examined	Blue eyes
At birth	1000	100 %
6th year	387	54 %
9th ,,	488	41 %
10th ,,	391	41 %
13th ,,	400	38 %
15th to 29th year	80	32 %
31st ,, 40th ,,	543	30 %
41st ,, 45th ,,	320	28 %
46th ,, 55th ,,	269	26 %

From these figures it is safe to conclude either that a tendency for the eye to darken exists through life, being most marked at birth and becoming less as time advances, or that death is strongly selective for this character. The former hypothesis is the more reasonable one. In later life a further alteration occurs which can be attributed to the loss of translucency of the stroma. It is obvious that eye-colour cannot be strictly regarded as a fixed character, so that some information as to age is necessary. In the adolescent period the change however is not marked.

There is of course no apparent reason why age of parent at birth should influence eye-colour, so that the result of an investigation of such a point must be *a priori* incapable of prediction.

The subject therefore under consideration resolves itself into the question of the existence rather than the nature of such a biological sequence.

For this purpose blue eyes were chosen and the number occurring in the various groups noted, all details as to shade or formation being ignored.

The data were collected in Middlesbrough and Barking through the opportunities afforded by the Medical Inspection of School Children. The first series are taken from Barking and consist of children in their 7th and 13th years, in the proportion of three to one. The mother's eye-colour was also taken so as to correct for any tendency of a particular type of eye-colour being more fertile and hence occurring more frequently than would be expected in a true random sample.

The tables are as follows:

TABLE II.

All Children.

	Child.		
	Blue	Not blue	Totals
	88	55	143
Mother: Blue	64	247	311
Not blue	152	302	454

$r = \cdot 5966 \pm \cdot 0253$.

TABLE III.

Age of Parent at Birth and Child's Eye-colour.

Age of parent at birth	Child's eye-colour		
	Blue	Not blue	Totals
16—20	4	7	11
21—24	38	67	105
25—28	36	74	110
29—32	29	66	95
33—36	23	47	70
37—40	8	23	31
41—44	10	14	24
45—48	3	3	6
Totals	151	301	452

$r = \cdot 0961 \pm \cdot 0334$.

$\eta = \cdot 1031$ and if corrected, indeterminate[1].

TABLE IV.

Age of Parent at Birth and her own Eye-colour.

Age of parent at birth	Mother's eye-colour		
	Blue	Not blue	Totals
16—20	5	6	11
21—24	35	63	98
25—28	25	76	101
29—32	26	59	85
33—36	20	41	61
37—40	8	19	27
41—44	6	6	12
45—48	1	4	5
Totals	126	274	400

$r = \cdot 0034 \pm \cdot 0337$.

$\eta = \cdot 0611$ and if corrected, indeterminate.

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[1] Pearson, K. (1912). A correction to be made to the Correlation Ratio. *Biometrika*, VIII. 254.

The coefficients are:

Age of mother at birth of child (1) and child's eye-colour (2)

$$r_{12} = \cdot 096 \pm \cdot 033.$$

Age of mother 9 years after birth of child (1) and her own eye-colour (3)

$$r_{13} = \cdot 003 \pm \cdot 034.$$

Eye-colour of mother (3) and child (2)

$$r_{23} = \cdot 597 \pm \cdot 025.$$

Making eye-colour of mother (3) constant then:—

Age of mother at birth of child (2) and child's eye-colour (1)

$${}_3r_{12} = \cdot 13 \pm \cdot 03.$$

This result suggests that children born of young mothers have a tendency to blue eyes, or that the change dependent on age is delayed. The values of the correlation ratios were found to be, mean of child's eye-colour for arrays of age at birth, $\eta = \cdot 103 \pm \cdot 033$, and if corrected becomes indeterminate; the same with means of mother's eye-colour $\eta = \cdot 061 \pm \cdot 034$, and is also indeterminate if corrected by Pearson's method.

To test the data for any bias in the method of collection, the eye-colour of the child was correlated with a different mother from its own.

The data are as follows:

TABLE V.

	Random mother		
Child:	Blue	Not blue	Totals
Blue	49	91	140
Not blue	103	207	310
Totals	152	298	450

$$r = \cdot 0362 \pm \cdot 0302.$$

TABLE VI.

Age of Random Parent at Birth and Child's Eye-colour.

	Random child		
Age of parent at birth	Blue	Not blue	Totals
16—20	2	4	6
21—24	20	33	53
25—28	21	30	51
29—32	10	24	34
33—36	12	12	24
37—40	8	7	15
41—44	2	6	8
45—48	1	0	1
Totals	76	116	192

$$r = -\cdot 0306 \pm \cdot 0477.$$

Age of random parent at birth (1) and child's eye-colour (2)

$$r_{12} = -\cdot 031 \pm \cdot 018.$$

Eye-colour random mother and child

$$r_{13} = \cdot 036 \pm \cdot 031.$$

Hence. Age of random parent at birth and child's eye-colour—parent constant

$${}_3r_{12} = -\cdot 03 \pm \cdot 05.$$

A result that suggests that the sample taken is not subject to any great error in collection.

In the second series of the data, collected in Middlesbrough, the age period was restricted to children of a particular age (8th to 9th year, *i.e.* born in year 1900). The sexes are dealt with separately and in each case the eye-colour of the mother is recorded, so as to remove any bias due to stock. The data are as follows:

TABLE VII.

Age of Mother at Birth of Child and her own Eye-colour.

Based on girls :

	Mother's eye-colour		
Age of mother	Blue	Not blue	Totals
20 and under	9	24	33
21—25	38	78	116
26—30	42	100	142
31—35	26	81	107
36—40	23	51	74
41 and over	3	13	16
Totals	141	347	488

$r = \cdot 0171 \pm \cdot 0303.$

$\eta = \cdot 118 \pm \cdot 029$ and if corrected $= \cdot 0607.$

TABLE VIII.

Based on boys :

	Mother's eye-colour		
Age of mother	Blue	Not blue	Totals
20 and under	7	15	22
21—25	35	71	106
26—30	36	97	133
31—35	21	64	85
36—40	13	43	56
41 and over	3	20	23
Totals	115	310	425

$r = \cdot 0494 \pm \cdot 0299.$

$\eta = \cdot 1592 \pm \cdot 0334$ and if corrected $= \cdot 1262.$

TABLE IX.

Age of Mother at Birth of Child and Child's Eye-colour (Girls).

Age of mother at birth	Child's eye-colour		Totals
	Blue	Not blue	
Under 20	12	21	33
21—25	56	60	116
26—30	58	84	142
31—35	41	66	107
36—40	30	44	74
41 and over	4	12	16
Totals	201	287	488

$r = \cdot 0355 \pm \cdot 0302.$

$\eta = \cdot 1289 \pm \cdot 0299$ and if corrected $= \cdot 0798.$

TABLE X.

Boys.

Age of mother at birth	Child's eye-colour		Totals
	Blue	Not blue	
20 and under	13	9	22
21—25	46	60	106
26—30	53	80	133
31—35	30	55	85
36—40	21	35	56
41 and over	5	18	23
Totals	168	257	425

$r = \cdot 0537 \pm \cdot 0301.$

$\eta = \cdot 2409 \pm \cdot 0324$ and if corrected $= \cdot 2151.$

TABLE XI.

Boys. 7—8 *years.*

Mother:	*Child.* Blue	Not blue	Totals
Blue	79	34	113
Not blue	88	223	311
Totals	167	257	424

$r = \cdot 5868 \pm \cdot 0216.$

TABLE XII.

Girls. 7—8 *years.*

Mother:	*Child.* Blue	Not blue	Totals
Blue	104	36	140
Not blue	103	240	343
Totals	207	276	483

$r = \cdot 6211 \pm \cdot 0193.$

From these figures for boys we have:

Age of mother at birth of child (1) and child's eye-colour (2)

$$r_{12} = \cdot 054 \pm \cdot 030.$$

Age of mother at birth of child (1) and her own eye-colour (3)

$$r_{13} = \cdot 049 \pm \cdot 03.$$

Child's and mother's eye-colour

$$r_{23} = \cdot 587 \pm \cdot 023.$$

Making mother's eye-colour constant

$${}_3r_{12} = \cdot 03 \pm \cdot 03.$$

The correlation ratios are:

Mean of eye-colour, child, for arrays of mother's age

$$\eta = \cdot 24 \pm \cdot 03 \text{ and if corrected} = \cdot 21.$$

Mean of eye-colour, mother, for arrays of her age

$$\eta = \cdot 16 \pm \cdot 03 \text{ and if corrected} = \cdot 13.$$

In the case of girls, we have:

Age of mother at birth of child (1) and child's eye-colour (2)

$$r_{12} = \cdot 036 \pm \cdot 030.$$

Age of mother at birth of child (1) and her own eye-colour (3)

$$r_{13} = \cdot 017 \pm \cdot 03.$$

Eye-colour, mother (3), eye-colour, child (2)

$$r_{23} = \cdot 621 \pm \cdot 019.$$

Making mother's eye-colour (3) constant

$${}_3r_{12} = \cdot 01 \pm \cdot 03.$$

The correlation ratios for arrays of mother's age at birth and mean of child's eye-colour

$$\eta = \cdot 13 \pm \cdot 03 \text{ and if corrected} = \cdot 08.$$

For arrays of mother's age and her own eye-colour

$$\eta = \cdot 12 \pm \cdot 03 \text{ and if corrected} = \cdot 06.$$

The values found in the south (Barking) are not identical with those found in the north (Middlesbrough). In the first, a small significant positive correlation exists; in the second series the value is still positive for both sexes, but it is not significant. The differences may be dependent on the fact that making the mother's eye-colour constant does not completely remove the effects that may arise from the different reproductive habits that are to some extent associated with eye-colour.

Although these results are of interest and suggest that some biological difference may exist of the nature suggested, they do not justify any definite assertion.

If we now consider age with respect to age of grandmother at birth of mother, taking the eye-colour of mother and child (the actual material is the same as in the previous series) we have:

TABLE XIII.

Age of Grandmother at Birth. Eye-colour of Mother and Child.

	Mother's eye-colour		
	Blue	Not blue	Totals
Mother born at 20 years and under	35	97	132
,, ,, 21—25	60	157	217
,, ,, 26—30	74	161	235
,, ,, 31—35	46	129	175
,, ,, 36—40	26	75	101
,, ,, 41 and over	15	38	53
Totals	256	657	913

$r = \cdot 002 \pm \cdot 021.$ $\eta = \cdot 06$ and if corrected, indeterminate.

TABLE XIV.

	Child's eye-colour		
	Blue	Not blue	Totals
Mother born at 20 years and under	16	39	55
,, ,, 21—25	73	149	222
,, ,, 26—30	78	179	257
,, ,, 31—35	47	145	192
,, ,, 36—40	36	94	130
,, ,, 41 and over	6	33	39
Totals	256	639	895

$r = \cdot 032 \pm \cdot 021.$ $\eta = \cdot 123$ corrected. $\eta = \cdot 098.$

From these observations we find:

Age of grandmother at birth of mother, and eye-colour of child

$$r = \cdot 032 \pm \cdot 021.$$

Age of grandmother at birth of mother, and eye-colour of mother

$$r = \cdot 002 \pm \cdot 021.$$

The correlation ratios are:

Mean of child's eye-colour for arrays of grandmother's age

$$\eta = \cdot 123 \pm \cdot 02 \text{ and if corrected} = \cdot 098.$$

Mean of mother's eye-colour for arrays of grandmother's age

$$\eta = \cdot 061 \pm \cdot 021 \text{ and if corrected, indeterminate.}$$

From this series it would seem as if there was no significant association between age of parent at birth and the eye-colour of an adult, hence from the previous observations, which suggest some association, it is possible that the rate at which the eye-colour develops from its initial blueness to its subsequent tint is modified, the change being more rapid in the later born. The subject could be more easily investigated by observing the alteration of tint in the first year of life. On the whole, however, we must regard the result of this investigation as purely negative.

Stature or Growth.

The relationship of growth or stature to age at birth has received considerable attention from different observers, amongst whom may be mentioned Gini, Marro, Stamini, Fourman, Budin, Ribemont, Schartzel, Hecker, and collected series of observations are also given in Vierordt's *Anatomische Daten u. Tabellen*, and in Prinzing's *Handb. d. medizinischen Statistik.*

The major portion of the work done by these observers has concerned itself with the development of the human infant at birth. Beyond the crude analysis of the figures little attempt has been made to separate the numerous factors that must necessarily play a more or less prominent part in modifying the development of the offspring. Analysis of the work of these observers shows that the variations are statistically significant, but concordance is wanting.

Thus according to age at birth, the mean weight and length of the infant increases, as age of mother at birth advances, whilst in association with order, the first or earlier born have the advantage. The influence appears to vary, according to whether the observations do or do not include those more comfortably placed economically. It is possible that the differences can be explained by the fact that the earlier born come in an undue proportion from the smaller families and those of the better social grades.

It has been shown by Arkle and many others that social position is associated with a better physical development so that should order be made constant, the apparent effect of age becomes more marked.

Further it has been suggested that the length of the puerpural period increases as age advances, hence the infant must not be regarded as starting life at the time of birth. Gini, comparing the expected weight of an infant making the observed order constant, shows that there is no significant variation with respect to age at birth, but his method does not allow for the physical development of the parents, and in so far as the earlier numbers are of a more heterogeneous type than the later, the process of calculation adopted cannot be said to have eliminated the disturbing factor of order.

In the following series of observations the age of both parents has been recorded. It was hoped that by making the age of mother constant for given age of father, that nutritive influences dependent on age of mother would be removed.

As will be seen no significant alteration is made in the final result. The material was collected in Middlesbrough under the Education Act, Administrative Provision, 1907.

TABLE XV.

Age of Father at Birth of Child and its Height.

Boys.

Inches

Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20—23	1	1	4	5	8	20	13	6	6	2	—	—	—	—	66
24—27	2	2	3	11	20	43	33	16	7	8	—	4	3	—	152
28—31	—	4	6	12	17	20	28	22	23	13	—	—	2	—	147
32—35	2	1	2	7	10	21	24	23	12	9	2	—	1	—	114
36—39	1	1	3	7	18	15	16	10	8	2	1	2	—	—	84
40—43	—	—	3	1	12	16	9	12	3	2	—	1	—	—	59
Over 43	—	1	2	2	3	6	9	4	4	1	1	—	—	—	33
Totals	6	10	23	45	88	141	132	93	63	37	4	7	6	—	655

$\sigma_{\text{height}} = 2{\cdot}0083$. $\sigma_{\text{age}} = 1{\cdot}2572$. $r = {\cdot}0213 \pm {\cdot}0261$. $\eta = {\cdot}0722 \pm {\cdot}022$.

TABLE XVI.

Age of Father at Birth of Child and its Height.

Girls.

Inches

Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20—23	—	3	2	8	7	11	21	9	5	—	—	—	—	—	66
24—27	—	5	10	10	12	29	19	14	14	6	5	1	—	—	125
28—31	4	1	1	6	23	19	41	18	5	7	1	—	—	—	126
32—35	2	1	5	11	12	27	26	14	9	2	2	—	—	—	111
36—39	1	2	5	6	11	15	13	10	8	4	—	—	2	—	77
40—43	1	1	—	6	6	18	12	9	3	2	1	2	—	—	61
Over 43	—	—	1	—	—	1	2	2	—	—	—	—	—	—	6
Totals	8	13	24	47	71	120	134	76	44	21	9	3	2	—	572

$\sigma_{\text{height}} = 2{\cdot}0054$. $\sigma_{\text{age}} = 1{\cdot}2743$. $r = {\cdot}0522 \pm {\cdot}0278$. $\eta_{\text{height}} = {\cdot}1263 \pm {\cdot}019$.

Boys and girls together, $r = {\cdot}0367 \pm {\cdot}0210$.

TABLE XVII.

Age of Mother at Birth of Child and its Height.

Girls.

	Inches														
Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	3	—	—	3	5	3	1	2	—	—	—		—	17
20—23	1	3	5	10	10	22	19	16	8	4	3	—	—	—	101
24—27	1	2	8	7	14	38	39	22	6	6	2	1	—	—	146
28—31	4	2	4	15	23	16	31	15	12	6	2	2	—	—	132
32—35	1	1	5	10	11	23	26	8	8	—	—	—	2	—	95
36—39	—	2	1	1	6	10	10	11	5	1	—	—	—	—	47
40—43	—	—	4	2	4	6	4	5	2	2	1	—	—	—	30
Over 43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals	7	13	27	45	71	120	132	78	43	19	8	3	2	—	568

$\sigma_{\text{height}} = 1{\cdot}9115$. $\sigma_{\text{age}} = 1{\cdot}2985$. $r = {\cdot}0675 \pm {\cdot}0287$. $\eta_{\text{height}} = {\cdot}1622 \pm {\cdot}015$.

TABLE XVIII.

Age of Mother at Birth of Child and its Height.

Boys.

	Inches														
Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	—	2	3	4	8	2		6	1	—	—	—	—	26
20—23	2	3	4	9	17	26	25	17	7	7	—	—	—	—	117
24—27	1	4	4	15	13	41	29	26	22	14	2	—	1	1	173
28—31	—	1	6	6	19	23	34	19	8	12	—	5	2	—	135
32—35	2	1	—	6	17	16	20	17	10	1	1	1	1	—	93
36—39	—	1	5	4	13	20	16	4	7	—	—	1	—	—	71
40—43	—	—	2	2	6	6	6	11	4	3	1	—	—	—	41
Totals	5	10	23	45	89	140	132	94	64	38	4	7	4	1	656

$\sigma_{\text{height}} = 2{\cdot}0419$. $\sigma_{\text{age}} = 1{\cdot}3261$. $r = {\cdot}0266 \pm {\cdot}0259$. $\eta = {\cdot}1178 \pm {\cdot}018$.

Boys and girls together, $r = {\cdot}0471 \pm {\cdot}0211$.

TABLE XIX.

Age of Father and Mother at Birth of Child.

Age of mother in years	Age of father in years						
	20 and under	21—25	26—30	31—35	36—40	41 and over	Totals
20 and under	7	26	7	4	—	—	44
21—25	6	92	76	17	7	2	200
26—30	—	18	123	92	17	6	256
31—35	—	1	21	78	41	21	162
36—40	—	—	4	4	61	51	120
41 and over	—	—	—	1	6	43	50
Totals	13	137	231	196	132	123	832

$\sigma_{\text{mother}} = 1{\cdot}2900$. $\sigma_{\text{father}} = 1{\cdot}3368$. $r = {\cdot}7858 \pm {\cdot}015$.

The coefficients evaluated are:

Age of father at birth of child (1) and its height (2)

$$r_{12} = \cdot 037 \pm \cdot 020.$$

Age of mother at birth of child (3) and its height (2)

$$r_{23} = \cdot 047 \pm \cdot 019.$$

Age of father (1) and age of mother (3)

$$r_{13} = \cdot 768 \pm \cdot 008.$$

Age of father and height of child, age of mother constant

$${}_3r_{12} = \cdot 001 \pm \cdot 021.$$

Age of mother and height of child, age of father constant

$${}_2r_{13} = \cdot 030 \pm \cdot 020.$$

η (mean of child's height for arrays of mother) $= \cdot 14 \pm \cdot 02$.

η (mean of child's height for arrays of father) $= \cdot 10 \pm \cdot 02$.

It would seem from these figures that there is no reason to believe that age *per se* has any effect on growth, but the environmental influence of the pre-natal period may have some effect. It is to be observed that the correlation ratios are significant, which suggests that the concomitant factors associated with age may be so arranged as to neutralise each other when viewed as a whole. It is necessary, therefore, to examine the matter in more detail before concluding that the effect of age at birth is negligible. This cannot be done without further material.

Age of Parent at Time of Birth and Intelligence.

The general literature on factors relative to intelligence is quite outside the scope of the present enquiry and that directly relevant to the subject matter seems to be limited to certain Italian writers, amongst whom are Lombroso, Orchansky, Marro, and Gini.

The conclusions arrived at by these writers are first that the influence of the mother's age predominates over that of the father's, and second that children born of younger parents seem to be more intelligent than those born of older parents. In the present paper the means of introduction were the same as in the previous sections, namely, through the Education Act of 1907. The standard of intelligence for parent and child was the ordinary scholastic test of proficiency; that is to say the age of the child and his or her standard; the mother's age on leaving school and her standard: and her age when the child under consideration was born were the details taken. It is to be noted that the object is to consider all children as produced by a uniform mother. To neutralise the disturbing factors noted above, we should reduce the data to such as would be produced by a common ancestry, which has not been done; so that we cannot claim that the possibility of race variations has been neutralised by removing any association that may be dependent on peculiarities of the mother. Two series were collected, firstly, a group of those in their 6th year (born 1903) and those in their 13th year (born 1895). No separation was made as to the sex in calculating coefficients. The data are as follows:

TABLE XX.

Age of Mother at Birth of Child and her own standard on leaving school (child in 5th year).

	Standard on leaving school				
	Standard VII		Other standards		
Age at birth of child	Boys	Girls	Boys	Girls	Totals
20 and under	4	6	10	16	36
21—25	34	30	45	48	157
26—30	43	45	56	74	218
31—35	24	9	35	38	106
36—40	7	11	21	25	64
41 and over	2	3	6	1	12
Totals	114	104	173	202	593

$r = - \cdot 2983 \quad \cdot 0251.$

TABLE XXI.

Age of Mother at Birth of Child and her own standard on leaving school (child in 13th year).

	Standard VII		Other standards		
Age at birth of child	Boys	Girls	Boys	Girls	Totals
20 and under	1	7	5	6	19
21—25	23	23	24	30	100
26—30	20	23	21	27	91
31—35	12	14	19	16	61
36—40	6	6	5	4	21
41 and over	5	6	2	2	15
Totals	67	79	76	85	307

$r = - \cdot 2609 \quad \cdot 0364.$

TABLE XXII.

Age of Mother at Birth of Child and its standard in 5th year.

Age at birth of child	Class I		Other classes		
	Boys	Girls	Boys	Girls	Totals
20 and under	9	20	3	3	35
21—25	58	65	28	20	171
26—30	88	81	30	32	231
31—35	47	46	19	15	127
36—40	20	34	12	8	74
41 and over	5	7	3	3	18
Totals	227	253	95	81	656

$r = \cdot 0525 \pm \cdot 0274$.

TABLE XXIII.

Age of Mother at Birth of Child and its standard in 13th year.

Age at birth of child	Standard of child				
	Standard VII		Other standards		
	Boys	Girls	Boys	Girls	Totals
20 and under	1	6	4	6	17
21—25	14	17	29	37	97
26—30	12	14	22	30	78
31—35	4	4	22	20	50
36—40	2	1	5	5	13
41 and over	1	2	2	1	6
Totals	34	44	84	99	261

$r = \cdot 067 \pm \cdot 0387$.

TABLE XXIV.

Standard of Mother on leaving school, and class of Child in 5th year.

Standard of mother	Class of child				
	Class I		Other classes		
	Boys	Girls	Boys	Girls	Totals
Standard VII	88	77	28	25	218
Other standards	122	148	61	46	377
Totals	210	225	89	71	595

$r = \cdot 534 \pm \cdot 0207$.

TABLE XXV.

Standard of Mother on leaving school, and standard of Child in 13th year.

Standard of mother	Standard of child Standard VII Boys and girls	 Other standards Boys and girls	Totals
Standard VII	43	34	77
Other standards	60	120	180
Totals	103	154	257

$r = \cdot331 \pm \cdot0354.$

TABLE XXVI.

Age of Mother when leaving school, and her standard (child in 5th year).

Age of mother	Standard VII	Other standards	Totals
14 years	149	114	263
13 ..	45	144	189
12 ..	25	85	110
11 ..	3	31	34
10 ..	—	8	8
9 ..	—	2	2
Totals	222	384	606

$r = + \cdot4975 \pm \cdot0231.$

TABLE XXVII.

Age of Mother when leaving school, and her standard (child in 13th year).

Age of mother	Standard VII Boys	 Girls	Other standards Boys	 Girls	Totals
14 years	16	26	21	27	90
13 ..	15	13	27	29	84
12 ..	1	5	21	24	51
11 ..	2	0	12	10	24
10 ..	—	—	—	3	3
9 ..			1	1	2
8 ..	—		—	1	1
Totals	34	44	82	95	255

$r = + \cdot4334 \pm \cdot0327.$

TABLE XXVIII.

Age of Mother when leaving school, and standard of Child in 5th year.

Age of mother	Class I	Other classes	Totals
14 years	192	64	256
13 ..	130	51	181
12 ..	81	29	110
11 ..	22	10	32
10 ..	6	1	7
9 ..	2	0	2
Totals	433	155	588

$r = + \cdot0129 \pm \cdot0275.$

TABLE XXIX.

Age of Mother when leaving school, and standard of Child in 13th year.

Age of mother	Standard VII	Other standards	Totals
14 years	26	49	75
13 ,,	31	39	70
12 ,,	10	34	44
11 ,,	5	12	17
10 ,,	2	—	2
9 ,,	—	1	1
8 ,,	1	—	1
Totals	75	135	210

$r = \cdot 0184 \pm \cdot 0477.$

TABLE XXX.

Age of Mother at Birth of Child and her age on leaving school (child in 5th year).

Age of mother at birth of child	Age of mother on leaving school						
	14 years	13 years	12 years	11 years	10 years	9 years	Totals
20 years and under	15	14	4	2	1	—	36
21—25	71	49	26	7	5	1	159
26—30	89	71	47	10	1	—	218
31—35	45	24	27	9	—	1	106
36—40	33	27	4	4	—	—	68
41 and over	8	—	2	2	—	—	12
Totals	261	185	110	34	7	2	599

$r = - \cdot 031 \pm \cdot 027.$

TABLE XXXI.

Age of Mother at Birth of Child and her age on leaving school (child in 13th year).

Age of mother at birth of child	Age of mother on leaving school						
	14 years	13 years	12 years	11 years	10 years	9 years	Totals
20 years and under	4	5	4	2	—	—	15
21—25	30	34	19	12	1	—	96
26—30	31	22	15	5	2	—	75
31—35	17	18	10	3	—	1	49
36—40	3	5	1	3	—	—	12
41 and over	4	2	1	—	—	—	7
Totals	89	86	50	25	3	1	254

$r = - \cdot 082 \pm \cdot 038.$

The values found for children in the 5th and 13th years were as follows:

		Children in	
		5th year	13th year
Age of mother on leaving school (1) and her own standard (2)	$r_{12} =$	·498 ± ·023	·433 ± ·033
Age of mother on leaving school (1) and class of child in 5th and 13th years (3)	$r_{13} =$	·013 ± ·028	·018 ± ·048
Age of mother at birth of child (4) and its class in 5th or 13th year (3)	$r_{43} =$	·053 ± ·027	·067 ± ·039
Age of mother at birth of child (4) and her own standard (2)	$r_{42} =$	− ·298 ± ·025	− ·261 ± ·035
Standard of mother on leaving school (2) and class of child in 5th or 13th year (3) ...	$r_{23} =$	·534 ± ·021	·331 ± ·035
Age of mother on leaving school (1) and age of mother at birth of child (4)	$r_{14} =$	− ·031 ± ·027	− ·082 ± ·038

The two series show a fair agreement, although the associations revealed are small. The following inferences may be drawn.

I. That there is a significant association between the age at which the mother left school and the standard she had attained. With respect to her child's intellectual standard, it is not significant.

II. That the age of the mother when the child was born is associated with her own intellectual grade, the sign being negative; that is, those who reproduce in late life are of a lower intellectual grade. With respect to the child's standard, the association is positive, but very small.

III. That the intellectual standards taken are highly correlated in mother and child, and on that account are probably a fair test of the point under investigation. It is worthy of note, that the association between mothers and children is greater in the fifth year than in the thirteenth year. This is due either to the fact that the latter group is selected from children of more careful parents than the former, or that children are promoted according to age rather than ability.

The intellectual grading of children in our elementary schools has been investigated statistically by Pearson and Jones (*Biometrika*, Vol. VII. p. 542). Mrs Frances Wood has also dealt with this subject in the Barking area, and she has kindly allowed me to use her results—as yet unpublished. The two investigations placed side by side are:

	Barking, North St School	Pearson and Jones
r, age and standard ... ... =	+ ·73	+ ·94
r, intelligence and standard ... ... =	+ ·40	− ·06
r, age and intelligence ... ... =	− ·215	− ·183
r, intelligence and standard with age constant =	+ ·83	

The value obtained for $_ar_{is}$ (+ ·83) points to a very definite system of promotion according to ability. Further $r_{is} = +$ ·40 would suggest that the more able children tended to pass into the higher classes, irrespective of age, for the correlation between age and intelligence is −·215.

The trend of these results rather suggests that promotion depends upon the personnel of the school, and on the accommodation. The former hardly needs comment. It is obvious that in a series of classrooms to hold 60 children and every classroom full, promotion according to age irrespective of intelligence must occur. On the other hand if classrooms accommodate 60 children and the average size of class is 40, grading according to intellect becomes possible. In the Middlesbrough area in which the data of this paper were collected, the overcrowding of the schools was considerable.

IV. The fourth value is what would be expected; that is, mothers who reproduce late, tend to have left school early.

We can now proceed to remove the disturbing factors, but it must be remembered that, with a mother of constant intelligence, racial effects have not been removed, but only reduced.

Making age at which the mother left school, and her standard constant (that is, considering the problem as if all children came from mothers of constant intelligence), then the correlations between age of mother at birth of offspring and its intellectual grade are, for children in the 5th year $r = ·32 \pm ·04$ and for children in the 13th year

$$r = ·17 \pm ·03.$$

It is seen that a crude correlation of ·05 is increased to approximately ·25 when the character examined is made constant for the female parent. The same point has been noted before, namely, that the effect of parental age can only be measured when the various compensating factors are removed.

The remaining partial coefficients are given in the following table.

	2nd order	
	Child in 5th year	Child in 13th year
$_{34}r_{12}$	·59 ± ·02	·45 ± ·02
$_{31}r_{42}$	·44 ± ·03	·30 ± ·03
$_{32}r_{41}$	·24 ± ·04	·06 ± ·03
$_{21}r_{43}$	−·38 ± ·03	·14 ± ·03
$_{24}r_{13}$	·32 ± ·04	·17 ± ·03
$_{14}r_{23}$	·65 ± ·02	·35 ± ·02

It is to be noted that the correlation between age of mother at birth and age of mother on leaving school, with other factors constant, is higher for children in the 5th year than in the 13th. This is an expected result, as reproduction in late life means a larger family, and the withdrawal from school at the earliest opportunity of children approaching the 13th year. It is obvious that data of this kind are subject to many curious influences which are extremely difficult to remove and, in fact, cannot be completely neutralised. It must therefore be with extreme hesitation that any inferences are drawn from the data collected in this way.

GENERAL CONCLUSIONS.

I shall now briefly summarise the conclusions I feel justified in drawing from the evidence submitted in the series of communications, of which the present is the fourth, reserving a full discussion for another occasion.

It appears, then, that as the age of the parent at the birth of the child increases, (*a*) the average length of life of the offspring decreases: (*b*) the fertility of the offspring increases: (*c*) the offspring react less characteristically to zymotic infections: (*d*) the proportion of males born increases: (*e*) the rate of change from congenital to ultimate type of eye-pigmentation of the offspring increases; (*f*) the intellectual grade of the offspring as defined by a scholastic standard rises.

Each of these conclusions depends upon the discovery of statistical constants significant with regard to an estimated error of sampling, but in no single case is the absolute intensity of the relation between the variables measured large, with two exceptions each of which is suspect, owing to the necessary ambiguity of the measures chosen by, or rather imposed upon, the investigator by the nature of the case.

In effect, the evidence that the populations of children born to parents of greater or less age are differentiated is inconclusive in any particular case taken by itself, but when we remember that a large variety of disparate attributes has been studied, that the particular fallacies inherent in each special investigation are likewise different, and that nevertheless all the results tend in the same direction, it is, I think, legitimate to infer that the populations are really differentiated.

Now we can suppose that the age of the parent may affect the characters of the offspring in the following ways.

Directly. In that (*a*) the primordial germ cells formed in late life partake of the senescence characterising the somatic cells, (*b*) the germ cell up to fertilisation is unaffected by senile somatic changes but thereafter suffers from nutritive effects dependent upon the soma.

Indirectly. In that the parental populations are differentiated, that those persons who bear or procreate children late in life are of a different type from others.

With respect to the second group of possibilities, we have already seen that in practice there is an economic differentiation which seriously complicates our analysis, for the more wealthy and educated classes marry later and cease to bear children earlier than the poorer members of the community. A good deal of statistical work in these papers has been undertaken with the object of eliminating this indirect factor, how far successfully is for the reader to judge, but the author recognises that the attempted neutralisation, particularly in the case of intelligence dealt with in the last part of the present paper, may well be deemed partly successful only.

Reverting to the direct effects, it may be said that although the Weismannian tradition, that of the inviolability of the germ plasm, has been notably weakened by the impact of hostile researches, yet even now the possibilities scheduled under (*b*) will seem to most of us the more inviting.

We may, however, assert that the (*b*) factors cannot suffice to account for all the facts. Thus, in all the quantitatively measurable characters studied, it has been found that the children of the younger or elder parents show changes in variability that cannot be attributed to maternal influence, which, of course, (*b*) covers completely. The data grouped in this way (taken from the peerage and referring to eldest born sons) are as follows:

Standard deviation of age of son at death, when born

before and including 31st year	32nd year or later
7·0865 ± ·1647	7·8033 ± ·1909

Diff. ·7168 ± ·2521. Ratio 2·84.

Age of father at death who reproduced

before and including 31st year	32nd year or later
7·0378 ± ·1327	6·3474 ± ·1514

Data with respect to the female parent are not forthcoming in the case of the peerage, but the variability in the number of children dying in a working class family is:

Standard deviation of number of children dying of mothers, born of grandparent

less than 30 years of age	more than 30 years of age
1·828 ± ·0344	1·888 ± ·0456

The difference is not significant and the suggestion is that the foregoing results can hardly be explained by reference to the female parent.

It may be of interest to examine the other characters dealt with from this point of view, taking growth, and dividing our data at the 30th year.

Standard deviation of height of mothers who reproduced a child

before and including 30th year	after 30th year
2·3550 ± ·0479	2·1326 ± ·0540

Height of children in 9th year—reproduced

before and including 30th year	after 30th year
2·3428 ± ·0479	2·4436 ± ·0618

Diff. ·1008 ± ·078. Ratio 1·4.

It is worthy of remark that the increased variability of the later born is itself a differentiation and a rather unexpected one. By the nature of the case the parents who reproduce late in life are a selection, a certain portion of the whole frequency of the parents being excluded. This kind of selection will tend to diminish the variability of the parental generation and to a less extent that of the filial generation. The stringency of the selection is not so intense nor the force of heredity so great in the case of the variables studied that we should expect any great reduction of variability; assuredly, however, we should not anticipate an increase which nevertheless we find.

There is however one apparent exception to the above. It is as follows (peerage data):

Standard deviation of number of offspring of son born

before 32nd year of father	after 32nd year of father
3·7127 ± ·0762	3·4561 ± ·073

Diff. ·256 ± ·091. Ratio 2·4.

This is contrary to what would have been expected as the increased variability of the life period of later born sons should lead to an increase in variability in the number of offspring produced. With respect to the female parent (working class data) the same trend is shown but the difference is not significant.

Standard deviation of number of children of daughter born

before 30th year of mother	after 30th year of mother
2·878 ± ·0499	2·7503 ± ·0572

Diff. ·12 + ·07.

This exception may be a special adaptation and not a true exception to what seems to be a general rule.

It agrees however with the following series in one respect, namely that an increase of the standard deviation is not accompanied by a corresponding change in the mean[1]. In these cases the characters of the parental generation could not be obtained.

Standard deviation of the age of attack of scarlet fever, of persons born

	before 30th year of mother	after 30th year of mother
	3·7072 ± ·0559	4·4227 ± ·0793
Mean	7·27 ± ·03 years	7·29 ± ·04 years

Standard deviation of the age of attack of diphtheria, of persons born

	before 30th year of mother	after 30th year of mother
	3·5635 ± ·0833	3·8769 ± ·1117
Mean	6·07 ± ·04 years	5·92 ± ·05 years

Standard deviation of the age of attack of small pox, of persons born

	before 30th year of mother	after 30th year of mother
	2·6977 ± ·1103	2·8933 ± ·1255
	—	—

With the steady improvement of the method of compiling and presenting anthropometric data consequent upon the enlargement of the scope of public health administration, we may look to the provision of material fit to provide a basis for a more complete and searching investigation than I have found possible. In the meantime these results will be of service to those who wish to inquire into a fascinating and, it may be, sociologically important subject.

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[1] In the cases of Scarlet Fever and Diphtheria, the coefficients of variation increase from 51·0 to 60·7 and from 58·7 to 65·5.

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SOME PROTEOLYTIC ANAEROBES ISOLATED FROM SEPTIC WOUNDS.

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(With 2 Text-figures.)

During four months' work with anaerobes, which were derived from wounds, a number of species have been noticed which appear to be hitherto undescribed. Two of these are proteolytic organisms and are not pathogenic to guinea-pigs when administered in large doses.

Owing to the extreme difficulty of getting pure cultures of anaerobes with ordinary technique, isolations were attempted by Miss M. Robertson and myself by means of Barber's method (*Philippine Journ. of Science*, vol. B IX. 1914, p. 307). Our results were very satisfactory. Instead of plating, isolations were made at random from the mixed cultures, some tubes receiving one bacillus and some several. The resulting cultures, if of interest, were treated in a similar manner. The type strains used for these descriptions were, to the best of my knowledge, derived from single bacilli isolated from apparently pure cultures, and they have behaved consistently in my hands.

ORGANISM I.

The first bacillus to be described has been isolated from two cultures. One case was that of a shrapnel wound of the maxilla which exuded a quantity of thick yellow pus. The sample was taken 20 days after the man was wounded and contained also *Bacillus sporogenes* (Metch.) and cocci. The second culture was taken from a pus wound located in the region of a tibia fractured by a high explosive shell. The sample was obtained 72 days after the injury occurred, and the wound had been treated with lotio rubra, permanganate, eusol, peroxide and saline. I found besides the organism in question cocci and end-sporing organisms with circular end-spores and bacilli of the morphology of *B. sporogenes* (both the ordinary variety and a smooth colonied variety) and of the morphology of *B. Hibler* IX. The organism described below was the dominant one in both cultures: two or three others showed, when old, forms morphologically similar to it. The type strain was derived from a single organism isolated in turn from a single bacillus culture from a glucose agar tube colony.

Staining and Morphology. The bacillus is usually 3—6 times as long as it is wide and it has rounded ends. It may or may not retain Gram's stain. Under uniform conditions it is consistently paler and more gram-negative than *B. sporogenes*. The spores are oval and subterminal, though a few forms are so short as to be clostridial. Sometimes in meat cultures forms with a spore at either end are common. Gram's stain is more easily washed out from the body of the bacillus than from the sporing end. The organism forms spores readily on most media. On agar plates uniform gram-negative bacilli are the rule. In old meat cultures free-swimming forms disappear, but the mass of the meat is inhabited by enormous pale gram-negative bacilli slightly tapering at the tip and bent in wide curves. This form is the most characteristic one of the species: similar forms occur on Dorset's egg medium. On gelatine it may grow as uniform gram-negative bacilli with pointed ends, spores being absent.

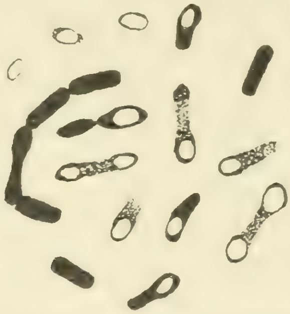
Fig. 1. Representative forms from a two day meat culture.

Motility. The organism is motile but not highly so.

Cultural Characters. *Meat*[1]. The growth on autoclaved meat is very characteristic. Twenty-four hours after inoculation there is usually heavy growth, the meat is pink, and some gas is present: the reaction is slightly acid. On re-incubation the meat settles more and more together with slight digestion, but it never disintegrates and never blackens. When pressed with a platinum loop it is soft and easily broken, but not pasty and not finely granular. On long incubation it becomes bright terra cotta in colour, sometimes with a faded grey layer on top. Blackening, the general assumption of a brown or grey colour, disintegrating digestion or a refusal to digest at all are indications of impurity; on the other hand, several varieties of contaminating organisms may be present without greatly affecting the appearance of the meat. There is a putrefactive odour which is not especially characteristic.

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[1] Autoclaved meat medium and alkaline egg medium are described by Miss Robertson in her paper, "Notes upon Certain Anaerobes derived from Wounds," *Journ. of Pathol. and Bact.* XX. 348.

Milk. In a hydrogen cylinder the litmus in milk is reduced if the organism grows; the milk is clotted *without* acid and slowly forms a soft irregular mass not torn by gas. Digestion of the clot begins after a few days and proceeds rather slowly; it is apparent from the yellowing of the liquid around the clot. This peculiar reaction has been consistent during long handling of the organism. The clotted milk placed on faintly blue litmus paper fails to turn it pink, and it is probable that the clotting is due to a weak rennet-like enzyme.

Alkaline egg. A fine coagulum is formed which renders the medium opaque.

Gelatine. The behaviour on gelatine, incubated at 37°, is rather irregular. Pure strains usually liquefy gelatine in two or three days although some gelatine tubes have remained unliquefied for two weeks. This irregularity is probably due to a difference in the quantity of the sowing used for the inoculation. There is a very misleading stage when a flake-like growth pervades the medium which stiffens as usual on cooling. On re-incubation for 24 hours the gelatine is liquefied.

Inspissated serum is attacked vigorously. Pitting occurs and the bases of the pits are white when many organisms are present. The surface becomes irridescent and sometimes brownish. Digestion is rapid at first but ceases before the medium is completely liquefied.

Dorset's egg medium is rendered soft and is cracked at the edges without liquefaction.

Ordinary broth and *glucose broth* are unsatisfactory media for this organism.

Action on carbohydrates. The organism was inoculated on serum litmus agar slopes containing various carbohydrates. It grew very well but failed to produce acid or gas in any case. The litmus was reduced and the mass of serum agar became somewhat pitted where the inoculation was the heaviest. The carbohydrates used were dulcite, inulin, glycerine, mannite, sucrose, glucose, maltose, laevulose and lactose. The strain used in these determinations was not the "single bacillus" strain.

Deep glucose agar tubes were excellent for the preliminary isolation of this organism. The colony in deep agar is at first round; later it takes the shape of a little worm, never growing more than 1·5 millimetres in length, and it is never woolly or radiate.

Growth on plates. This organism is easily grown on glucose agar plates. They should be dried for 20—40 minutes in the incubator before they are inoculated, and they must be exhausted and incubated immediately after inoculation. Colonies appear in 48 hours and at this period they are water-clear and very tiny. They are irregular in outline with a definite concentric ring arrangement, the middle portion being slightly granular under the microscope and the outer portion translucent. If the agar is too wet the growth will spread over the plate with fucus shaped projections.

After four days' incubation the colonies are easily visible through the substrate. They may be as much as two millimetres in diameter, are bluish grey in colour, and look to the naked eye as though they were speckled with white. Microscopically they are irregular edged and are no longer clear but are granular and wavy in texture.

Finally, these colonies are never woolly and never have fine processes: they are always minute and rarely round. They do not dig into the substrate nor are they raised or boss-like. They do not show up on the first day nor are they white or yellow.

Pathogenicity. Two cubic centimetres of a two days' broth culture of this organism failed to cause more than a slight swelling when injected intramuscularly into a guinea-pig.

Temperature of growth. This organism grows excellently at 37° and well also at 27° but at the latter temperature it does not start to grow as quickly. A newly inoculated meat tube shows no change at room temperature (*c.* 20°), but an old one goes on settling and reddening.

Agglutination. Bacillus I does not agglutinate with the anti-sporogenes serum of Weinberg.

Morphologically this organism would be easily confounded with *B. sporogenes* which it much resembles. The appearance of a young culture in meat medium resembles that of the acid producers, especially that of *B. perfringens*, but the organism bears so little morphological resemblance to any of those with which we are familiar that there is no danger of confusion.

ORGANISM II.

This is a typical proteolytic organism which is capable of splitting some of the single sugars as well as proteins. It has been isolated from two cultures in this laboratory. One was taken from a very bad case of gas gangrene the material being sent from France by Capt. J. W. McNee, R.A.M.C.

The wound culture contained cocci, anaerobic bacilli of the morphology of *B. sporogenes*, *B. perfringens*, *B. Hibler* IX, an organism with circular end-spores and three apparently undescribed organisms. Organism II was first cultivated by Miss Robertson and handed over to me to describe. It was also isolated by myself from a culture from a bad gas gangrene case of Dr Brookes in the Horton War hospital. The infected leg was amputated and the patient ultimately recovered.

The other organisms found in this wound were cocci and anaerobes of the morphology of *B. perfringens*, *B. Hibler* IX, *B. sporogenes* and end-sporing organisms with circular end-spores. The strain used for this description was obtained from Capt. McNee's case and was derived from an apparently pure culture from a colony on an agar plate. Two bacilli isolated from this culture by Barber's technique gave a pure culture from which in turn a single bacillus was isolated for the type strain.

Staining and Morphology. The organism is more uniform in its morphology than most anaerobes. In the non-sporing state, the bacillus resembles exactly *B. perfringens*, being chunky and having very square ends. It takes Gram's stains so deeply that it usually appears black; however in a preparation from an agar slope many pale forms are seen among the dark ones. A young culture always contains many sporing individuals; after four days these may be no longer in evidence. The spores are usually central but they may be terminal. They are large in proportion to the size of the bacillus and usually markedly square at the ends. They do not bulge the sides of the bacillus to any appreciable extent and are usually clear and definite in outline. Two spores are never found in one bacillus. The size of the organism varies greatly; in a fresh meat culture the bacilli are all very large; a four days' culture has large and small forms. On broth, alkaline egg, serum and gelatine the bacilli are all much smaller than on meat. In old meat cultures and in old serum cultures long thick crooked gram-positive rods occur which are often granular.

Motility. The organism is non-motile.

Cultural Characters. *Meat.* The reaction on this medium is very characteristic. The meat retains the colour and form that it had before inoculation but it settles somewhat in the tube. A little gas forms in the first hours and then disappears; it is usually not noticed at all. When pressed with the loop the meat in old cultures is easily disintegrated but is not granular or brittle. It never becomes really digested and does not spontaneously disintegrate. The liquid above the meat is yellow and perfectly clear. A putrefactive odour is noticeable.

Milk. This organism does not clot milk or render it acid. It deposits the casein in microscopic granules and then digests it very rapidly, three days' incubation being sufficient to destroy the casein. The litmus is reduced.

Alkaline egg. A very soft coagulum is formed.

Gelatine is liquefied in less than 24 hours.

Inspissated serum. The growth on this medium is fairly plentiful. Digestion begins after a few days' incubation but stops before it has proceeded far owing to the fact that the organism ceases to grow.

Ordinary broth is a good medium.

Action on carbohydrates. A series of serum litmus agar slopes containing various carbohydrates were inoculated and the organism grew well. It produced both acid and gas on glucose, maltose and laevulose media, and acid alone on glycerine medium. Neither acid nor gas was produced on dulcite, inulin, mannite, sucrose or lactose media.

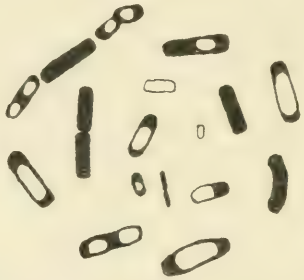

Fig. 2. Representative forms from a two day meat culture.

On *glucose agar plates*, treated as described for the preceding organism, the growth was very abundant. Pale bluish colonies were formed, usually irregular in outline but with smooth edges. The colonies were entirely homogeneous and of even thickness. The largest ones were two and three millimetres in diameter.

In *deep glucose agar tubes* tiny colonies are formed: they are irregular in outline but they are not radiate nor worm-shaped nor woolly. After five days' incubation they average ½ millimetre in diameter and none of them is more than ¾ millimetre in diameter.

PATHOGENICITY. 2 c.cm. of a 48-hour broth culture failed to produce any pathological symptoms in a guinea-pig when injected into the muscles of the thigh.

TEMPERATURE OF GROWTH. The organism will grow excellently at 37° and also at *c.* 27° but is rather slow in starting to grow at the latter temperature.

AGGLUTINATION. Bacillus II does not agglutinate with the antisporogenes serum of Weinberg.

It is of interest to note that the organism bears a close morphological resemblance to *B. perfringens.* Some of its sporing forms are microscopically almost indistinguishable from a violently pathogenic gas forming organism isolated by the author from a human case. This pathogenic organism corresponds closely to vibrion septique, Pasteur.

IDENTIFICATION. I think it is probable that Bacillus II is the same organism as *B. bifermentans sporogenes* of Tissier and Martelly (*Ann. de l'Institut Past.* 1902, p. 894). These authors say however that *B. bifermentans sporogenes* resembles *B. perfringens* and that their rôle seems to be identical. Whether this statement is due to a misunderstanding of the rôle of *B. perfringens*, I do not know, but I can draw no parallel between my organism and *B. perfringens* except the morphological one and the fact that they both split certain sugars. The putrefactive action of *B. bifermentans sporogenes* is far less than that of organism II, the former liquefying gelatine in a month the latter in a night: the former first changing the appearance of milk after five days, the latter showing digestion after a few hours and completely destroying the casein in a few days. These differences are perhaps to be accounted for by different media and technique. Recent experience with anaerobes inclines me to suggest that the strains of Tissier and Martelly were composed of the same organism as mine plus a small proportion of a member of the acid forming group which decreased the proteolytic power of the predominant organism.

Bacillus II resembles morphologically *B. sporogenes foetidus* of Choukewitch (*Ann. de l'Inst. Past.* March and April, 1911) but the latter organism is feebly motile and does not form gas in deep glucose agar.

Clostridium foetidum of Liborius (*Zeitschr. f. Hyg.* I. 1886, p. 160) and *Clostridium foetidum cernis* of Salus (*Archiv f. Hyg.* LI. 118) do not resemble organism II morphologically and they are highly motile.

The "pseudo-oedem bacillus" of Liborius is very meagrely described: it somewhat resembles my organism but is said to form at times two spores in one bacillus.

I wish in conclusion to express my heartiest thanks to Miss Muriel Robertson for her interest in this work and to Dr Harden of the Lister Institute for so kindly affording me the courtesies of the laboratory.

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XXV. FURTHER OBSERVATIONS ON THE INFLUENCE OF PHENOL AND OF CRESYLIC ACID ON THE CONCENTRATION OF ANTITOXIC SERA BY THE BANZHAF (1913) PROCESS.

By ANNIE HOMER.

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(*Received July 27th, 1917.*)

It has been already pointed out [Homer, 1917] that in the Banzhaf [1913] process for the concentration of antitoxic sera the difficulties encountered in filtration can be obviated by (1) an adjustment of the reaction of the sera, and (2) by the addition of phenol, cresylic acid or "trikresol" to the ammonium sulphate mixtures previous to their being heated.

The first of these methods of procedure requires more care and attention than can always be afforded in a laboratory where concentrations are carried out on a commercial scale. The second is much simpler and gives good results as may be seen from the details of the further experimental work which has been carried out in this connexion.

Phenol and its homologues not only increase the heat denaturation of the serum proteins but also so influence the precipitability of the particles of heat denaturated protein that they are retained with the First Fraction precipitate. This phenomenon, presumably brought about by virtue of the effect of the above mentioned substances on the surface tension of the protein particles in colloidal solution, leads to the production of a final product which is clear and which filters readily through filter candles.

Experience has shown us that, in order to obtain the desired results, the percentage of these substances added to the serum, must fall within certain limits. Should the amount not reach the lower limit then the end products are more unsatisfactory than if no phenol, etc., had been added. Should the upper limit be exceeded then some of the antitoxin is transferred to the First Fraction Precipitate from which it cannot be recovered by extraction with 30 % of saturation with ammonium sulphate: for this purpose prolonged extraction with brine is necessary.

Experimental concentrations illustrating this point, each with not less than one litre of plasma, were carried out as follows:

To oxalated plasma were added 2 % of solid sodium chloride and one-fifth of its volume of water[1]. To the diluted plasma were then added varying amounts of phenol or of cresylic acid followed by the volume of a saturated solution of ammonium sulphate necessary to bring the ammonium sulphate content of the mixture to 30 % of saturation.

The phenol or cresylic acid-serum-ammonium sulphate mixtures were distributed in stoppered glass jars and placed in a bath containing water at a temperature of from 62 to 64°. The temperature of the serum mixtures gradually rose to 60°. As soon as this was reached the jars were taken from the bath and allowed to cool to 45° and filtered (First Fraction Precipitate).

The precipitates were washed with a solution containing ammonium sulphate to the extent of 30 % of saturation. The filtered washings were added to the main bulk of the filtrates which were then brought up to 50 % of saturation with ammonium sulphate. The ensuing precipitate (Second Fraction Precipitate) was filtered, pressed and dialysed in the usual way. To the residue from dialysis was added 1 % of sodium chloride and 0·35 % of cresylic acid.

A comparison was then made between the various end products thus obtained as regards their clearness and their tendency to become cloudy on standing. At the same time data were furnished as to the percentage removal of the total serum proteins and also as to the percentage of the total antitoxic units of the original serum appearing in the end product in those cases in which the higher percentages of phenol and cresylic acid had been used. The antitoxic content of the end products, where lower percentages of these substances had been used, were not estimated in the published series of experiments owing to our shortage of experimental animals. From other work we have satisfied ourselves that in the latter cases there is no appreciable loss of antitoxin during the process of concentration.

The results which have been embodied in the accompanying table (p. 281) indicate that:

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[1] Banzhaf recommends that the plasma be diluted with one half its volume of water previous to the addition of the ammonium sulphate. However, in our routine work, we no longer consider the dilution of the plasma of any advantage, for it is our experience that the heat denaturation and precipitation of the serum proteins is a factor of the reaction and not of the dilution of the sera.

In my experimental work the dilution of the plasma with one-fifth its volume of water is a matter of personal convenience for the reading of the scale of the Zeiss Refractometer.

(1) Cresylic acid has a greater effect on the heat denaturation and precipitation of the heat denaturated proteins than has phenol. Moreover, the effect of the former takes place at a lower temperature than that of the latter.

The end products obtained from concentrations in which 0·30 % of cresylic acid had been added to the plasma were more clear and filtered better than when 0·50 % of phenol had been used.

(2) The addition of progressively increasing amounts of phenol or of cresylic acid to samples of the same sera previous to the heating of the serum mixtures was followed by a correspondingly increased percentage removal of the serum proteins of the original plasma.

(3) For the production of clear and readily filterable end products which would remain clear after filtration it was found necessary to add a minimum of 0·30 % of cresylic acid to the diluted plasma.

With a smaller amount of cresylic acid it was found that the end products were cloudy and far more unsatisfactory than if the Banzhaf process had been carried out without the addition of this substance.

(4) While the percentage of cresylic acid may be increased to 0·35 %, it is inadvisable to go beyond this limit, as the ensuing increased denaturation induces a transference of antitoxin to the First Fraction Precipitate from which it can only be recovered by long extraction with brine.

(5) The degree of concentration is considerably increased by the addition of 0·30 to 0·35 % of cresylic acid to the plasma previous to concentration.

Thus, whilst by the Banzhaf (1913) technique during the process of concentration, there is usually a 4 to 5 times increase in potency per cc., with the above modification the potency per cc. is increased 7 to 8·5 times[1].

In view of the above experimental data the concentration of routine batches of 50 and 100 litres of sera was conducted in the same way and similar results ensued. The protocol of the concentration of a 50 litre batch of antitetanic serum is given below.

T. R. 65.

Volume of oxalated plasma taken	50 litres
Volume of water added	10 ,,
Weight of NaCl added	1200 g.
Volume of cresylic acid shaken with 1 litre of water and added to the diluted plasma	180 cc.
Volume of a saturated solution of ammonium sulphate added	25,700 cc.

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[1] The potency can be still further increased (viz. to the order of 10 times) by making the precipitation limit for the Second Fraction Precipitate 45 per cent. of saturation with ammonium sulphate instead of 50 per cent. as hitherto used [Homer, 1917].

Temperature to which the cresylic-plasma-ammonium sulphate mixture was just heated ... ... 60°

Percentage increased precipitation of the serum proteins induced during the heating of the mixtures to 60° 28

Original Plasma	**Final Product**
Volume, 50 litres	Volume, 6·5 litres
Potency per cc., 200 (slightly +)[1]	Potency per cc., 1650
Protein content, 8·26 %	Protein content, 18·6 %

We thus see that, in the above concentration:

(1) there has been a removal of 70 % of the total proteins of the sera taken for concentration,

(2) the loss of antitoxic units during the process has been negligible,

(3) the potency of the serum has been increased eight times.

The final product was clear and limpid. On dilution with saline it showed no trace of the presence of a colloidal suspension of euglobulin or of heat denaturated protein. Its filtration through filter candles proceeded with ease and the filtered product showed no signs of becoming cloudy on standing.

From these results it will be seen that, by the addition of 0·30 to 0·35 % of cresylic acid to the diluted plasma before concentration by the Banzhaf method. the degree of concentration and the percentage removal of the serum proteins thereby induced are of the same order as those obtained by the method recently published from these laboratories [Homer. 1916]. Such a simple modification of the technique of the former process entailing the production of a highly concentrated product will probably be welcomed by those laboratories in which my double heating process has not yet been adopted.

SUMMARY.

The difficulties encountered in the Banzhaf (1913) technique and the tendency of the end products to become cloudy can be obviated by the addition of 2 % of sodium chloride and of 0·30 to 0·35 % of cresylic acid to the plasma previous to the heating of the plasma-ammonium sulphate mixtures.

There will not be undue loss of antitoxin in presence of the specified amounts of cresylic acid provided that:

(*a*) the addition of 2 % of sodium chloride be made a matter of routine,

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[1] The experimental guinea-pig to which 100 M.L.D.'s of U.S.A. Standard Tetanus Toxin and 1/2000 cc. of the above plasma had been given died within 120 to 136 hours after the injection.

A comparison between the end products obtained from the concentration of antitoxic sera by the Banzhaf (1913) *Process and by a modification of the same process in which to the plasma diluted with one-fifth its volume of water have been added varying amounts of phenol and of cresylic acid previous to the heating of the serum mixtures.*

Experimental No.	Percentage of protein in the original plasma	Percentage of phenol or of cresylic acid added to the *diluted* plasma	Percentage increase in the precipitation of the soluble proteins during the raising of the temperature of the phenol or cresylic acid serum ammonium sulphate mixtures to 56	60	Percentage of the total proteins of the original plasma appearing in the final product	Percentage of the total antitoxic units of the original plasma appearing in the final product	Increased potency per cc. over that of the original plasma	Appearance of the final product standing in bulk in the cold room within (*a*) 24 hours, and (*b*) three months of being taken from the dialysing bags: (*a*) after 24 hours	(*b*) after 3 months	The effect of dilution of the end product with twice its volume of saline
Antidiphtheritic serum										
49 *a*	7·81	0·00 phenol	2·1	5·2	64·5	—	—	slightly turbid	turbid	opalescent
49	7·81	0·35 ,,	11·0	17·0	45·0	>84*	4·5	,, ,,	,,	slightly opalescent
50	7·81	0·50 ,,	15·5	20·8	40·0	>83*	5·5	very slightly turbid	slightly turbid	very slightly opalescent
51	7·81	0·35 cresylic acid	24·6	26·0	30·0	82	7·5	clear	clear	clear
Antitetanic serum										
75 *a*	6·49	0·00 cresylic acid	—	2·1	65·0	—	—	slightly turbid	turbid	opalescent
75	6·49	0·15 ,, ,,	—	15·0	45·0	—	—	,, ,,	,,	,,
80	6·49	0·20 ,, ,,	—	17·0	36·7	—	—	,, ,,	,,	slightly opalescent
81	6·49	0·25 ,, ,,	—	22·5	35·0	88·0	6·5	,, ,,	slightly turbid	,, ,,
82	6·49	0·30 ,, ,,	—	25·0	28·1	88·0	8·5	clear	clear	clear
Antidiphtheritic serum										
85	7·61	0·00 cresylic acid	—	3·0	63·1	—	—	clear	turbid	opalescent
86	7·61	0·25 ,, ,,	—	26·0	42·9	—	—	turbid	,,	,,
87	7·61	0·30 ,, ,,	—	30·0	37·1	—	—	clear	clear	clear

* Not tested further on account of the shortage of experimental guinea pigs. For the same reason tests were not carried out on 49 *a*, 75 *a*, 75, 80, 85, 86, and 87 as we have (in other work) satisfied ourselves that with as much as 0·5 and 0·35 % of cresylic acid there is no undue loss of antitoxin provided that the temperature of the serum mixture be not raised beyond 60°.

(*b*) the mixtures be neither heated beyond 60° nor allowed to remain at this temperature for more than two or three minutes.

The addition of the cresylic acid has considerably enhanced the degree of concentration that can be obtained by the Banzhaf method. It has also increased the percentage removal of the serum proteins.

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XXVI. A NOTE ON THE USE OF INDICATORS FOR THE COLORIMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF SERA.

By ANNIE HOMER.

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(*Received Aug. 27th, 1917.*)

During the progress of a series of investigations on the concentration of antitoxic sera it was found necessary to adjust the reaction of the sera to certain fixed values in order to get concordant and consistent results. In the experimental work, owing to lack of data with regard to the reliability of indicators in the presence of the serum proteins, the hydrogen ion concentration of the sera was determined by the electrical method. Since the latter method cannot be conveniently used in a general routine laboratory, experimental work was undertaken to ascertain whether determinations of sufficient accuracy for practical purposes could be made by the colorimetric method.

In this connexion to several samples of different sera were added varying quantities of acid or of alkali. The hydrogen ion concentration $[H^{\cdot}]$ of each of the samples was estimated by the electrical method and the values thus found were compared with those obtained by the colorimetric method using the stated indicators and Sörensen's standard solutions in a Walpole's colorimeter, the colour system being as follows:

Eye.

Left	Right
10 cc. serum + x drops of Indicator	10 cc. serum + *no* Indicator
10 cc. water + *no* Indicator	10 cc. Sörensen's standard solution + x drops of Indicator

Source of Light.

In the colorimetric determination of the values for the [H˙] care must be taken to use a constant amount of the specific indicator. It is a matter of experience that, both with the standard solutions and with the test sera, the intensity of the colour reaction at a definite hydrogen ion concentration can be varied by alterations in the amount of indicator added, so that, even with indicators which may prove sufficiently reliable for use with sera, unless the amount of indicator be kept constant, neither consistent nor comparable results will be forthcoming.

For my purpose litmus, methyl violet, methyl orange and Henderson and Forbes' indicator were useless with serum as I was unable to match the two colour systems. In these cases the colour reaction between the serum and each of the above-mentioned indicators seemed to me to be different from that between the indicator and the standard solutions.

With *p-nitrophenol* and *tropaeolin OOO*, it was found that, although it was possible to match the two colour systems, the colour changes were neither sufficiently sharp nor in sufficient contrast to the colour of the serum itself for me to obtain satisfactory readings.

With *phenolphthalein* the colorimetric determinations gave values not sufficiently near those obtained by the electrical method to be of any service even for approximate values for routine work. Thus, the colour reaction between the indicator and serum corresponding to that with the standard solutions at P_H 8·25 was not evidenced until the true reaction of the serum had been brought to values of the order of P_H 10·5.

On the other hand, contrary to expectation, *neutral red* proved sufficiently reliable for routine determinations with sera. It was found that with the addition of five drops (Pasteur capillary pipette) of a 0·05 % solution of this indicator in alcohol to 10 cc. of the Sörensen's standard solutions the useful range was between P_H 5·6 and 7·8. The same amount of indicator was then added to 10 cc. of samples of the different sera and the colour reaction thus produced was matched with the colour scheme obtained by viewing the standard solutions and indicator through serum in the colorimeter: in serum the useful range was between P_H 5·9 and 7·6. The results thus obtained for the values of the [H˙] of the sera together with those obtained by the electrical method have been embodied in Table I.

TABLE I.

A comparison between the values obtained for the hydrogen ion concentration (P_H) of various sera by the electrical method and by the colorimetric method, using neutral red as the indicator.

[To 10 cc. of the test solutions were added five drops of a 0·05 % solution of neutral red in alcohol.]

Plasma No.	Percentage of protein in the sample of plasma taken	P_H of the plasma determined by the colorimetric method	P_H of the plasma determined by the electrical method
1	6·73	7·6	7·7
1 *a*	4·92	7·14	7·14
2	6·16	6·98	7·02
2 *a*	4·62	between 6·98 and 6·81	6·82
2 *b*	4·62	6·60	6·40
3	6·16	5·91	6·29
4	8·12	7·35	7·46
4 *a*	6·09	between 7·64 and 7·35	7·50
4 *b*	4·06	7·34	7·40
5	6·09	5·91	6·02
5 *a*	4·06	5·91	6·02
6	6·56	7·34	7·40
6 *a*	6·56	5·90	5·92
6 *b*	4·92	6·97	6·98
6 *c*	4·92	6·60	6·40

A study of Table I shows that the [H·] of sera can be determined between P_H 5·9 and 7·6 with sufficient accuracy for practical purposes by the colorimetric method using neutral red as indicator.

The studies of Sörensen and Palitzch [1910] have shown the value of *a-naphtholphthalein* as an indicator. It was tried with sera as its useful range, using three drops of a 0·10 % solution with the standard solutions, was from P_H 7·6 to 8·5.

From the data given in Table II it will be seen that the determinations by the electrical method are consistently higher than those by the colorimetric method using this indicator. Thus, the slightest tinge of green colorimetrically corresponding to that for P_H 7·6 with the standard solutions does not show until the true value for P_H is of the order of 8·5. However, with this indicator the "buffer" action of the proteins is not sufficiently marked to be too serious a drawback to its use for routine work. Thus, it was found that, if to 10 cc. of the serum containing three drops of the solution of *a*-naphtholphthalein ammonia was added until the appearance of the faintest green tinge in the serum was detected, the reaction of the serum thus treated was approximately P_H 8·5.

TABLE II.

A comparison between the values for the P_H *of various sera obtained by the electrical method and by the colorimetric method, using α-naphtholphthalein as the indicator.*

[To 10 cc. of the test solutions were added three drops of a 0·10 % solution of the indicator in alcohol.]

Plasma No.	Percentage of protein in the plasma	P_H determined by the colorimetric method	P_H determined by the electrical method
7	6·73	7·64	8·29
7 *a*	4·92	7·64	8·04
8	8·12	7·86	8·65
8 *a*	6·09	7·64	8·44
8 *b*	4·06	7·64	8·55
8 *c*	2·03	7·65	8·60
9	6·56	7·64	8·27
9 *a*	4·92	7·64	8·48
9 *b*	4·92	8·04	8·71
10	6·16	7·64	8·52
10 *a*	4·62	7·64	8·45
10 *b*	4·62	7·86	8·25

For routine work in which a value of about P_H 8·0 was desired it was found that the following procedure would approximately give the desired result: To an aliquot part of the serum was added the necessary volume (x cc.) of a standard solution of ammonia to bring the $[H^{\cdot}]$ to the value P_H 7·6, using neutral red as the indicator. To a similar volume of serum was added the necessary volume (y cc.) of the ammonia solution to bring the $[H^{\cdot}]$ to the value P_H 8·5 (faint green tinge with α-naphtholphthalein).

The addition of $\frac{x+y}{2}$ cc. of the standard solution of ammonia to the stated volume of serum brought the $[H^{\cdot}]$ approximately to the value of P_H 8·0. This is a purely empirical arrangement which has given results of sufficient accuracy for routine work for the reason that, while an excess of alkali is undesirable, slight variations in the reaction round about P_H 8·0 are not followed by the marked variations in the heat denaturation of the serum proteins so characteristic of sera more acid than P_H 5·5.

It was next desired to find reliable indicators for the determination of the $[H^{\cdot}]$ of sera more acid than could be tested by neutral red. For this purpose, owing to the unreliability of litmus, methyl orange, methyl red, Henderson and Forbes' indicator, and also to my inability to match the colour reaction with *p*-nitrophenol, resource was had to the sulphonephthalein indicators, the use of which has been so strongly advocated by Mansfield Clark and Lubs [1915]. The brilliancy of the colour reactions of these substances suggested that besides possibly furnishing indicators for the desired acid ranges they might also replace the indicators already in use on the alkaline side.

In this connexion, the following indicators in 0·05 % alcoholic solution, were used.

	Useful range P_H
Thymol-sulphonephthalein*	8·0–9·6
o-Cresol-sulphonephthalein*	7·2–8·8
Phenol-sulphonephthalein	6·8–8·4
Dibromothymol-sulphonephthalein*	6·0–7·6
Dibromo-*o*-cresol-sulphonephthalein*	5·2–6·8
Methyl red	4·4–6·0
Tetrabromophenol sulphonephthalein	3·0–4·6

* Samples of these indicators were very kindly sent to me by Dr Mansfield Clark as some delay was experienced in obtaining the chemicals necessary for their preparation.

The useful range of each indicator was tested against Sörensen's standard solutions which were also checked by the electrical method. A comparison was then made between the values of the [H·] of the various sera obtained by the colorimetric method using the above mentioned indicators and also by the electrical method. The data with regard to these observations have been embodied in Table III.

Thymol-sulphonephthalein was found to be unreliable with serum. Thus, the colour reaction corresponding to P_H 8·3 with the standard solutions did not appear in the serum until the value P_H 10·3 had been reached.

The colour changes with *o-cresol-sulphonephthalein* in serum could not be detected by me with sufficient ease for its adoption in routine work.

The colour reactions of *phenol-sulphonephthalein* and of *bromothymol-sulphonephthalein* in serum could be readily matched throughout their useful ranges with the standard solutions, but, the values for the [H·] of the sera thus determined were consistently lower than the true values determined by the electrical method. As it was found that, in sera containing from 4 to 10 % of protein, these deviations from the true values were constant for the same worker, use was made of these indicators for the determination of the [H·] of sera between the ranges P_H 6·8 and 9·0 as follows.

To the test serum were added six drops of a 0·05 % solution of the indicator. The colour reaction was then matched against standard solutions containing

TABLE III.

A comparison between the values obtained for the P_H *of various sera by the electrical method and by the colorimetric method using the indicators of the sulphonephthalein series.*

Plasma No.	Percentage of protein in the plasma	Indicator used	P_H of the plasma determined by	
			the colorimetric method	the electric method
11	8·12	Thymol-sulphonephthalein	8·03	10·34
12	6·16	,, ,,	8·03	10·21
13	6·56	,, ,,	8·03	10·33
14	4·40	,, ,,	8·03	10·55
15	6·73	Phenol-sulphonephthalein	6·81	7·65
15	6·73	,, ,,	7·14	8·00
15	6·73	,, ,,	7·34	8·25
15	6·73	,, ,,	7·64	8·55
15	6·73	,, ,,	7·86	8·70
15	6·73	,, ,,	8·03	8·97
16	4·92	,, ,,	7·64	8·43
17	6·16	,, ,,	7·64	8·46
18	8·12	,, ,,	7·64	8·25
19	6·09	,, ,,	7·86	8·70
20	4·06	,, ,,	7·44	8·27
20 *a*	4·06	Bromothymol-sulphonephthalein	6·23	6·86
21	6·09	,, ,,	6·46	7·31
22	4·92	,, ,,	6·46	7·58
23	4·40	,, ,,	6·46	7·38
24	8·12	,, ,,	6·81	7·86
24	8·12	,, ,,	7·10	8·10
24	8·12	,, ,,	7·30	8·66
25	6·97	,, ,,	7·30	8·60
26	9·90	,, ,,	7·30	8·70
27	6·73	Methyl red	4·98	4·00
28	4·92	,,	6·00	5·24
29	8·12	,,	5·60	4·80
30	8·12	Bromo-*o*-cresol-sulphonephthalein	5·2	5·14
31	6·09	,, ,,	5·2	5·06
32	6·56	,, ,,	5·2	5·14
33	4·40	,, ,,	5·2	5·23
34	6·73	,, ,,	5·4	5·50
35	7·56	,, ,,	5·4	5·58
36	8·12	,, ,,	5·4	5·65
37	6·56	,, ,,	5·4	5·32
38	6·73	Bromophenol-sulphonephthalein	3·0	2·90
39	8·12	,, ,,	3·0	2·87

a corresponding amount of indicator, the colour produced with the latter being viewed through serum. The value thus obtained was transmuted to the true value according to the following table:

Phenol-sulphonephthalein		Bromothymol-sulphonephthalein	
Value for P_H found by the colorimetric method	True value for P_H of the serum (approximately)	Value for P_H found by the colorimetric method	True value for P_H of the serum (approximately)
6·81	7·65	6·23	6·86
7·14	8·00	6·46	7·40
7·34	8·25	6·81	7·80
7·64	8·55	7·10	8·10
7·86	8·70	7·30	8·60
8·03	8·95		

For the colorimetric work with serum, I found it more easy to match the yellowish green to blue colour reactions of bromothymol-sulphonephthalein than the orange to reddish purple colour reactions of phenol-sulphonephthalein. For this reason in routine work the reaction of sera was approximately adjusted to the value P_H 8·0 by the addition of ammonia until the colour reaction of bromothymol-sulphonephthalein with the serum matched that with a standard solution at P_H 7·1, the latter being viewed through serum in the colorimeter. However, although fairly consistent results were obtained, I found that I was not always as successful with bromothymol-sulphonephthalein as with α-naphtholphthalein, the sensitiveness of my colour vision to the delicate colour changes of the former being very much influenced by personal fatigue.

With *dibromo-o-cresol-sulphonephthalein* and serum it was found somewhat difficult to match the various shades of purple through the ranges of the indicator. However, in practice, it was an easy matter to bring the serum to the degree of acidity at which the indicator showed a slight greenish tinge, *i.e.* just before the acid limit of the useful range of the indicator was reached. The reaction of the sera so adjusted was found to be approximately P_H 5·5.

If acid were added to serum containing bromo-*o*-cresol-sulphonephthalein until the final slight green tinge had just disappeared it was found that approximately the value P_H 5·2 had been reached.

For the adjustment of the sera between the values P_H 5·5 and P_H 4·5 the following arbitrary method was adopted which gave results of sufficient accuracy for routine work, provided the colorimetric determinations were made by the same observer.

Sera containing from 5 to 8 % of protein were brought to P_H 5·5 (circ.) by the addition of the requisite amount (x cc.) of acetic acid to a stated volume of serum containing a fixed amount of indicator until only the faintest tinge of green was detected. Since the colour of serum is affected by its acidity or alkalinity, the comparison in all cases was made with the same volume of serum containing no indicator but to which had been added a corresponding amount of acetic acid. It was found that the further addition of acetic acid to the serum beyond this point gave results of the following order:

Volume of *N* acetic acid (or equivalent) added to 100 cc. of the serum	P_H of the acidified serum (approximately)
x cc.	5·5
$x+0·5$ cc.	5·4
$x+1·0$	5·3
$x+1·5$	5·2
$x+2·0$	5·1
$x+2·5$	5·05
$x+3·0$	4·9
$x+4·0$	4·7
$x+5·0$	4·6

The value of such an arbitrary method depends on the acuteness of the colour vision of the individual and, for this reason, in order to obviate the personal error in obtaining the determination of the initial value depending on the colour tint, which, for me, approximated to the value P_H 5·5, it must be urged that each investigator should work out his own table.

Methyl red proved to be unsatisfactory with serum, as the colorimetric determinations gave results not sufficiently near those obtained by the electrical method to justify its use in routine work, slight changes in the reaction of sera on the acid side of P_H 5·5 leading to marked variations in the heat denaturation and precipitation of the serum proteins.

Difficulty was also experienced in matching the purple colour of *bromophenol-sulphonephthalein* in serum. It was however found that, with the sera taken, the faint green tinge just before the useful range of the indicator on the acid side was passed (*i.e.* at P_H 3·0) could be regulated with approximate accuracy.

During the course of the investigation it was found that dilution of the serum was followed by sharper changes in the colour reactions throughout the useful ranges of all the above-mentioned indicators. But, even where the dilution had been of the order of 1 in 1, there was no appreciable variation in the values obtained for P_H by the colorimetric method.

SUMMARY.

During the course of the investigation it has been demonstrated that the hydrogen ion concentration of sera can be determined, with sufficient accuracy for routine work, by the colorimetric method with the use of certain indicators.

It was my experience that:

1. Neutral red gave reliable results with serum throughout the ranges P_H 5·9 to 7·6.

2. The results obtained with α-naphtholphthalein were consistently higher than the true values. Thus, the colorimetric determination of the values for P_H 7·6 was not given until the reaction of the sera had been adjusted to the value P_H 8·5.

3. By a combination of the use of neutral red and of α-naphtholphthalein the adjustment of routine batches of sera to P_H 8·0 can be arranged with sufficient ease for practical purposes.

4. Of the indicators of the sulphonephthalein series thymol-sulphonephthalein and *o*-cresol-sulphonephthalein proved unsatisfactory with serum. Phenol-sulphonephthalein and bromothymol-sulphonephthalein gave results which were consistently higher than the true values. Bromo-*o*-cresol-sulphonephthalein could be used with ease for P_H 5·4 and 5·2 while bromophenol-sulphonephthalein was satisfactory for values about P_H 3·0.

5. By a purely arbitrary arrangement based on my own observations, I was able to adjust the reaction of sera approximately to values between P_H 7·6 and 8·95 with the use of phenol-sulphonephthalein; to values between P_H 6·8 and 8·6 with the use of bromothymol-sulphonephthalein and to values between P_H 5·5 and 4·5 with the use of bromo-*o*-cresol-sulphonephthalein.

6. In view of the personal error involved in the determination of colour reactions with sera it is advisable that each worker using the colorimetric method should ascertain the degree of approximation of his own values to the true values determined by the electrical method.

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XXVII. ON THE INFLUENCE OF THE HEAT DENATURATION OF PSEUDOGLOBULIN AND ALBUMIN ON THE NATURE OF THE PROTEINS APPEARING IN CONCENTRATED ANTITOXIC SERA.

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In previous communications [Homer, 1916, 1917, 1, 2] it has been demonstrated that, as a result of the heating of serum, considerable changes are effected in the precipitability of the serum proteins by 30 % of saturation with ammonium sulphate, the extent of the changes being a function of the reaction of the serum.

The practical application of these results to the concentration of antitoxic sera has led to the preparation of end products (the residue from the dialysis of the protein precipitated between 30 and 50 % of saturation with ammonium sulphate) containing from 20 to 30 % only of the total proteins of the original serum. These results show an improvement on those obtained by the methods hitherto published.

Recent work, however, has shown that the final products obtained by methods involving a heat denaturation of the serum proteins contain albumin as well as pseudoglobulin and, moreover, that the relative amount of albumin increases with the extent of the heat denaturation of the proteins.

The presence of albumin in the final product is to be avoided for two reasons: the one that, since the antitoxin is associated with the pseudoglobulin and not the albumin constituent of the serum proteins, the presence of albumin in the final product must lower the degree of concentration; the other that the inclusion of albumin may be undesirable from the clinical point of view [Dale and Hartley, 1916].

Before attempting to ascertain whether an alteration of the precipitation limits for the Second Fraction precipitate would obviate the precipitation of heat denaturated albumin with the pseudoglobulin, it was necessary to furnish experimental evidence with regard not only to the extent to which the denaturation of the serum proteins was affected by the time of heating but also to the extent to which the denaturation of the individual serum proteins was affected by the reaction of the serum.

Owing to the necessity for the immediate practical application of the results of the investigation to routine work, the experiments have, for the present, been confined to a study of the behaviour of pseudoglobulin and of albumin in this latter respect.

A. THE INFLUENCE OF THE TIME OF HEATING OF SERA ON THE EXTENT OF THE HEAT DENATURATION OF THE SERUM PROTEINS.

During the progress of the experimental work involved in the study of the influence of the reaction of sera on the extent of the heat denaturation of the serum proteins it was noticed that, with alkaline sera, the changes were a gradual function of the time of heating while, with acid sera, the changes were complete within a comparatively short time.

In order to furnish detailed evidence on this point the following experiments were undertaken. Antitoxic serum to which had been added 2 % of sodium chloride was diluted with one-third its volume of water. To separate volumes, each of 100 cc. of the diluted plasma, were added varying amounts of acid and of alkali and the hydrogen ion concentration $[H^{\cdot}]$ of the various experimental liquids was measured by the electrical method. The stoppered bottles containing the experimental liquids were then placed in a water bath at 57·5° and were heated for several hours at that temperature.

Samples of the experimental sera, previous to their being heated, were made 30 % of saturation with ammonium sulphate and filtered. The protein content of the filtrates was measured by means of the Zeiss refractometer. Similar measurements were made with samples of the heated liquids withdrawn from time to time from the various bottles of sera in the bath. From the data thus obtained was calculated the percentage increase in the precipitability of the serum proteins after the heating of the sera for the stated periods of time.

The results have been incorporated in Table I and indicate that:

(1) During the heating of alkaline sera the extent of the heat denaturation gradually increases with the prolongation of the time of heating.

The maximum value is obtained after four hours' heating at 57·5°.

(2) In the acid sera the extent of the heat denaturation has practically reached its maximum value after one hour at the specified temperature.

(3) The nature of the changes involved in the alkaline sera is different from that in the acid sera, for at no time during the heating of the alkaline sera does acidification of the clear heated liquids to a value P_H 5·0 produce the precipitate of denaturated protein so characteristic of heated acid sera.

During the course of the work it has also been observed that both with acid and alkaline sera the maximum effects are produced:

(*a*) more quickly if the temperature to which the serum is heated be raised beyond 57·5°, and

(*b*) more slowly if the sera be heated at temperatures lower than 57·5°.

Table I. *The influence of the time of heating the sera at* 57·5° *on the extent of the heat denaturation of the serum proteins.*

[Protein content of the plasma = 7·15 %.]

Volume of acid or of alkali added to 100 cc. of the diluted plasma	P_H of the diluted plasma measured by the electrical method	Percentage increase in the precipitability of the serum proteins by 30 % of saturation with ammonium sulphate after heating the serum at the specified temperature for a period of				
		1 hr	2 hrs	3 hrs	4 hrs	6 hrs
4 cc. *N* NH_3	9·4	23·1	37·2	—	44·0	45·0
2	9·1	23·0	32·0	—	40·0	40·0
1	8·8	22·2	30·0	—	40·0	40·0
0·6	8·2	17·7	27·0	—	34·6	35·6
0·5 cc. *N* HAc	7·4	12·0	17·0	—	22·0	22·6
0·75	7·1	9·0	11·0	—	16·0	16·8
2·5	5·9	13·0	12·9	13·0	—	—
3·0	5·7	13·0	16·6	16·6	—	—
3·5	5·5	15·0	16·0	16·0	—	16·0
4·0	5·3	20·0	23·5	24·2	—	—
4·5	5·1	30·0	32·8	33·0	—	33·0
5·0	5·0	34·8	40·0	40·0	—	—
6·0	4·8	60·9	67·5	67·0	—	—
6·5	4·7	64·6	79·8	79·8	—	80·0

These results will be of service to those engaged in the concentration of antitoxic sera. They show that, in order to get the maximum effect, in the case of sera more alkaline than P_H 7·4 the time of the preliminary prolonged heating of the serum at 57·5° must not be less than four and need not be greater than six hours after the liquid has reached the specified temperature. In the case of acid sera the full effect is produced within from one to two hours of the temperature reaching 57·5°.

To what extent variations in the temperature at which the serum is heated affect the order of the maximum value for the heat denaturation of the serum proteins at a particular $[H^{\cdot}]$ of the serum is under investigation.

B. THE EFFECT OF CHANGES IN [H·] ON THE DENATURATION OF PSEUDOGLOBULIN INDUCED DURING THE HEATING OF ITS SOLUTIONS AT A TEMPERATURE OF 57·5° FOR A PERIOD OF SIX HOURS.

A solution of pseudoglobulin was prepared as follows:

To four litres of antidiphtheritic serum were added two litres of water and six litres of a saturated solution of ammonium sulphate. The liquid was allowed to stand for one hour and was then filtered. The precipitate was washed with four litres of a half-saturated solution of ammonium sulphate and, after being thoroughly drained, was thrown into four litres of a saturated solution of salt containing an excess of solid salt. The salted liquid was allowed to stand for two days, care being taken to macerate the precipitate thoroughly. The liquid was then filtered and to the filtrate was added 0·25 % of glacial acetic acid. The ensuing precipitate was filtered, pressed and, after the addition of 3 % of solid crystallised sodium carbonate, was dialysed. The residue from dialysis was diluted until the protein content was of the order of 8 %, and 1·5 % of solid salt was then added.

Aliquot parts of the solution were measured into stoppered glass bottles; varying amounts of acid and of alkali were added and the [H·] of each of the liquids was determined by the electrical method. The bottles containing the liquids were then heated in a water bath at a temperature of 57·5° for a period of six hours. The protein content of the unheated liquids and also of the filtrates from samples of the unheated liquids brought up to 30 % of saturation with ammonium sulphate were measured by means of the Zeiss immersion refractometer. The values thus obtained were compared with the corresponding values for the heated liquids. From the data thus furnished were calculated the percentage precipitation of pseudoglobulin itself and also the percentage increased precipitability of the pseudoglobulin by 30 % of saturation with ammonium sulphate induced during the heating of the pseudoglobulin solution. The results which have been incorporated in Table II (p. 297) have also been plotted in Figure 1, Curves 1 and 2.

The appearance of the heated liquids was characteristic throughout the ranges of acidity and alkalinity investigated. On the alkaline side the liquids were clear or were characterised by an opalescence. On the acid side the liquids were converted during the heating into thin suspensions, the semi-solid consistency of which became more marked as the acidity increased.

From a study of the data given in Table II, Column *A*, it will be seen that the conversion of soluble pseudoglobulin into insoluble protein was of the order of 5 % or less in the liquids of which the reaction lay between the ranges P_H 5·5 and 9·7. On the acid side of P_H 5·5 there was a marked precipitation of insoluble protein; thus, at P_H 5·34 it was of the order of 17·9 % while at P_H 4·6 it was of the order of 78 %.

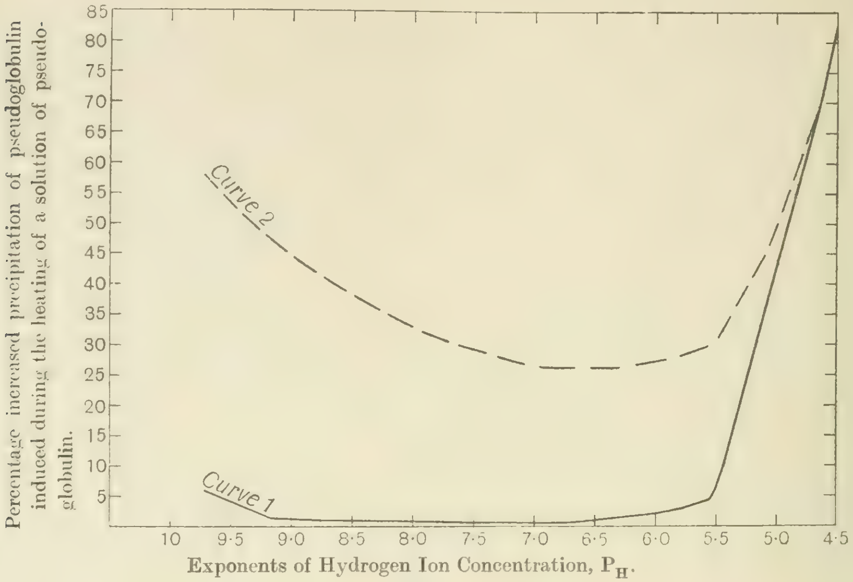

Fig. 1

Curve 1 ——— represents the influence of changes of reaction on the conversion of soluble pseudoglobulin into an insoluble condition during the heating of solutions of pseudoglobulin at 57·5° for 6 hours.

Curve 2 – – – represents the accompanying percentage increased precipitability of the heat-denaturated pseudoglobulin.

The data given in Column *B*, Table II show that there was, throughout the whole range investigated, a marked increase in the precipitability of the serum proteins by 30 % of saturation with ammonium sulphate: the extent of the increase was a function of the reaction of the liquid.

The increased precipitability was least between the range P_H 5·5 and 8·0. On either side of these limits the rate of increase was appreciable: the gradient for the increase being more gradual on the alkaline than on the acid side. As the degree of alkalinity decreased from P_H 9·7 to 8·0 the increased precipitability decreased from 57·6 to 32·6 %; between P_H 8·0 and 5·5 it varied from 25 to 30 %, while between P_H 5·3 and 4·6 it rapidly rose from 49·0 to 75·5 %.

TABLE II. *Showing the influence of the hydrogen ion concentration on the denaturation of pseudoglobulin induced during the prolonged heating of a 7·95 % solution of pseudoglobulin at a temperature of 57·5 for 6 hours.*

Volume of normal acid or alkali added to 100 cc. of the pseudoglobulin solution	P_H of the solutions measured by the electrical method	Appearance of the heated liquids	*A* Percentage conversion of soluble into insoluble protein during the heating of the pseudoglobulin solutions	*B* Percentage increase in the precipitability of the pseudoglobulin by 30 % of saturation with ammonium sulphate
5·0 cc. *N* NH_3	9·72	clear	5·5	57·6
4·0	9·51	,, ...	5·5	57·0
3·0	9·40	,, ...	4·2	50·4
2·0	9·30	,, ...	1·3	44·0
1·50	9·03	very slightly opalescent ...	1·3	43·7
1·00	8·63	slightly opalescent	1·3	42·4
0·50	7·97	,, ,, ...	0	32·6
0·25	7·40	opalescent	0	29·2
0·00	6·81	very thin emulsion	0	25·6
0·50 cc. *N* HAc	6·43	thin emulsion, filtrate opalescent	1·3	26·3
1·00	5·99	thin emulsion, filtrate slightly opalescent	4·2	27·6
1·60	5·34	thick emulsion, filtrate clear	17·9	40·0
2·50	5·06	,, ,, ,,	43·0	48·2
3·30	4·80	,, ,, ,,	60·9	58·9
4·25	4·64	almost solid, filtrate clear ...	66·0	64·2
5·0	4·60	,, ,, ,, ...	78·0	75·5

C. THE EFFECT OF CHANGES IN [H·] ON THE HEAT DENATURATION OF ALBUMIN INDUCED DURING THE HEATING OF ITS SOLUTIONS AT 57·5° FOR A PERIOD OF SIX HOURS.

The solution of albumin was prepared as follows:

The filtrate from the precipitated globulins (ante, p. 295) was saturated with ammonium sulphate and the ensuing precipitate was filtered off. The precipitate was washed from the filter paper into three litres of a half-saturated solution of ammonium sulphate. The liquid after standing for twenty-four hours was filtered, and the filtrate was saturated with ammonium sulphate and allowed to stand for two days at room temperature. The precipitated albumin was filtered off, thoroughly drained, pressed and dialysed until the residue in the bags was practically free from sulphate. The residue from dialysis was diluted until the protein content was of the order of 6 % and 1·5 % of solid sodium chloride was then added.

To aliquot parts of the above solution of albumin were added varying amounts of acid and alkali so as to bring the [H·] of the series of experimental liquids within the ranges P_H 9·7 and 4·5: the [H·] of each of the solutions being measured by the electrical method previous to the liquids being heated at a temperature of 57·5° for a period of six hours.

The protein content of each of the above liquids and of the filtrates from

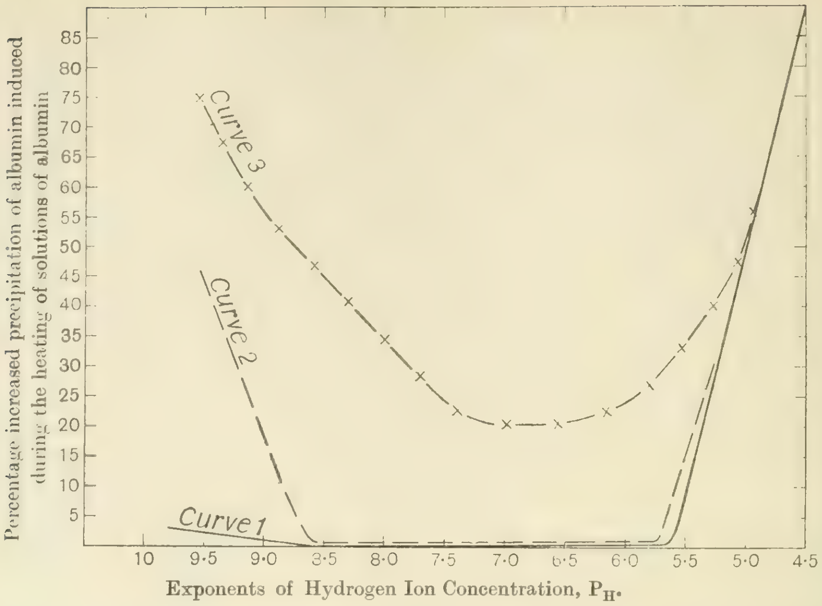

Fig. 2

Curve 1 ——— represents the influence of changes of reaction on the conversion of soluble albumin into an insoluble condition during the heating of solutions of albumin to 57·5° C. for 6 hours.

Curve 2 — — — represents the accompanying percentage increased precipitability of the heat denaturated albumin by 30 % of saturation with ammonium sulphate.

Curve 3 —×—×— represents the accompanying percentage increased precipitability of the heat denaturated albumin by 50 % of saturation with ammonium sulphate.

them made 30 % and also 50 % of saturation with ammonium sulphate were estimated before and after their having been heated.

The data thus obtained have been incorporated in Table III and have been plotted in Curves 1, 2 and 3 in Figure 2.

From a study of these data it will be seen that, as in the case of solutions of pseudoglobulin, the heated liquids on the alkaline side were either clear or else showed only a slight opalescence, whereas on the acid side the liquids were converted into thin suspensions the consistency of which became more markedly solid as the degree of acidity increased.

From the results given in Column *A* of Table III it will be seen that the actual conversion of soluble albumin into an insoluble condition was less than 5 % between the ranges P_H 9·5 and 5·5. On the acid side of P_H 5·5 it rapidly rose from 6·0 % at P_H 5·5 to 57·6 % at P_H 4·87 and to 100 % at P_H 4·40.

From the results given in Column *B* of the same table it will be seen that the precipitability of albumin by 30 % of saturation with ammonium sulphate is not appreciably affected during the heating of solutions of which the

TABLE III. *Showing the influence of the hydrogen ion concentration on the denaturation of albumin induced during the prolonged heating of a 5·20 % solution of albumin at a temperature of 57·5° for a period of 6 hours.*

Volume of normal acid or alkali added to 100 cc. of the solution of albumin	P_H of the solutions measured by the electrical method	Appearance of the heated liquids	Column *A* Percentage conversion of soluble into insoluble protein during the heating of the albumin solutions	Column *B* Percentage increase of the precipitability of the albumin by 30 % of saturation with ammonium sulphate	Column *C* Percentage increase of the precipitability of the albumin by 50 % of saturation with ammonium sulphate
4·3 cc. *N* NH_3	9·53	slightly cloudy	2·2	46·0	75·0
3·0	9·11	,, ,, ...	1·0	36·7	58·0
2·2	8·70	clear	0·0	8·0	50·0
1·00	8·41	,, ...	0·0	0	44·0
0·50	7·81	,, ...	0·3	0	31·5
0·25	7·40	,, ...	0	0	22·7
0·00	6·90	,, ...	0	0	21·3
0·70 cc. *N* HAc	6·27	slightly opalescent, filtrate opalescent	0	0	26·1
1·40	5·53	cloudy, filtrate opalescent ...	6·0	14·2	34·2
2·20	5·18	thin emulsion, filtrate clear ...	30·0	36·7	44·0
3·0	4·87	thick ,, ,, ...	57·6	55·0	57·0
6·0	4·52	,, ,, ,, ...	89·0	Too thick to be measured in a pipette	Too thick to be measured in a pipette
8·5	4·40	almost solid, filtrate clear ...	100		
6·0 cc. *N* H_2SO_4	3·40*	,, ,, ,, ...	100		

* Opalescent after the addition of acid.

reaction falls between the limits P_H 8·4 and 5·5. On either side of these limits there is a rapid rise in the precipitability of the albumin.

In Column *C* have been given the data with regard to the increased precipitability of the solution of albumin by 50 % of saturation with ammonium sulphate. It will be seen that the increased precipitability of the albumin is a function of the $[H^\cdot]$ of the solutions. Thus, as the $[H^\cdot]$ increased from P_H 9·5 to 7·8 so the increased precipitability decreased from 75 % to 31 %; between the values of P_H 7·8 and 5·5 it varied from 20 to 30 % while on the acid side of P_H 5·5 the percentage increased precipitability was practically equivalent to the percentage conversion of soluble into insoluble protein.

The results recorded above for pseudoglobulin and for albumin respectively show certain similarities with those previously obtained from corresponding experiments with antitoxic sera [Homer, 1917, 1]. Thus it was noticed that:

(1) The extent of the heat denaturation of the individual proteins, pseudoglobulin and albumin, is a function of the $[H^\cdot]$ of the solutions.

(2) There is coincidence between the curves representing the conversion of soluble into insoluble protein during the heating of whole serum, of solutions of albumin and of pseudoglobulin respectively.

(3) The curve for the increased precipitability of albumin by 50 % of saturation with ammonium sulphate has a configuration very similar to those for the increased precipitability of pseudoglobulin and of the proteins of whole serum respectively by 30 % of saturation with ammonium sulphate.

(4) The values for the increased precipitability of the serum proteins by 30 % of saturation with ammonium sulphate are somewhat lower than those for solutions of pseudoglobulin at the same [H˙]. This is probably due, partly to the diluent effect of the accompanying albumin in whole serum and partly to the "buffer" action of the latter.

(5) In all cases the minimum change takes place between the ranges P_H 5·5 and 8·0 and there is coincidence of the curves on the acid side of P_H 5·5.

(6) The changes taking place during the heating of alkaline sera and of alkaline solutions of pseudoglobulin and of albumin are not of the same type as those induced in the acid solutions, for acidification of the heated alkaline liquids to P_H 5·0 does not produce the thin suspension so characteristic of the heated acid liquids.

(7) The increased precipitability of the heated alkaline sera and of alkaline solutions of albumin and of pseudoglobulin only attains its maximum value after several hours' heating. On the other hand the changes in the acid liquids are practically complete within one hour's heating at the specified temperature.

From the work of Chick and Martin [1910, 1911, 1912, 1, 2] it was anticipated that while a certain similarity would exist between the configuration of the curves for the heat denaturation of pseudoglobulin and of albumin respectively at hydrogen ion concentrations on either side of their isoelectric points, there would be a shift in the position of corresponding portions of the curves due to the fact that the isoelectric point of pseudoglobulin is at P_H 5·52 while that of albumin is at P_H 4·70. Curiously enough not only was the region of least change the same in both cases, viz., from P_H 5·5 to 8·0, but there was absolute coincidence of the curves on the acid side of P_H 5·5.

These phenomena present points of interest in connexion with the views held both as to the justification for regarding the protein constituents of serum as well defined entities and as to the possible conversion of pseudo- into euglobulin and of albumin into pseudoglobulin.

The results of an investigation of the nature of the heat denaturated proteins obtained from albumin and pseudoglobulin will be dealt with in a later paper.

D. THE PRECIPITATION OF HEAT DENATURATED PSEUDOGLOBULIN AND HEAT DENATURATED ALBUMIN WITH THE FIRST AND SECOND FRACTION PRECIPITATES.

From a consideration of the above results it is obvious, that in sera which have been subjected to prolonged heating the First and Second Fraction precipitates respectively comprise the various proteins in proportions which are somewhat different from those in the corresponding precipitates in the unheated serum.

An investigation was therefore undertaken in order to furnish data with regard to the composition of the First and Second Fraction precipitates in heat denaturated sera throughout the ranges of acidity and alkalinity previously considered above in sections A, B and C.

The wider aspect of the problem has not yet been completed but, in view of the need for the immediate application of my results to routine concentration work, data are being published with regard to the precipitability of heat denaturated pseudoglobulin and albumin by ammonium sulphate from solutions the reaction of which had been adjusted to that of the routine batches of sera taken for concentration. The application of those results to the routine work has led to the production of end products in which the proportion of albumin has been lessened while the degree of concentration has been increased.

Solutions of pseudoglobulin and of albumin (prepared as in B and C), previous to their being heated to a temperature of 57·5° for six hours, were adjusted to about P_H 8·0. Samples of the heated and of the unheated liquids were made varying percentages of saturation with ammonium sulphate and were then filtered, the protein content of the filtrates being estimated by means of a Zeiss refractometer. From the data thus obtained was calculated the percentage conversion of pseudoglobulin and of albumin respectively from the emulsoid to the suspensoid condition induced by the addition of the various amounts of the electrolyte to the heated and to the unheated solutions.

The results obtained with pseudoglobulin have been embodied in Table IV. They show that in the unheated solutions the extent of the precipitation of pseudoglobulin increases with the concentration of ammonium sulphate, the change being complete at about 50 % of saturation with the electrolyte. In the heated solutions the precipitability of the protein by concentrations of ammonium sulphate beyond 20 % of saturation is considerably enhanced, the precipitation being practically complete at 42·5 % of saturation.

TABLE IV. *Showing the extent to which the precipitability of pseudoglobulin by ammonium sulphate is affected by the prolonged heating of its solution at 57·5° for 6 hours.*

[Percentage of pseudoglobulin in original solution = 7·10.]

P_H of the solution = 8·0.

Percentage of saturation with ammonium sulphate	Residual percentage of pseudoglobulin in solution in the filtrates from the ammonium-sulphate-pseudoglobulin mixtures		Percentage precipitation of pseudoglobulin by the ammonium sulphate in the	
	(*a*) before heating	(*b*) after heating	(*a*) unheated liquids	(*b*) heated liquids
20	6·92	6·77	2·5	4·6
25	6·77	5·65	4·6	20·5
28	6·67	4·56	6·0	35·9
30	6·04	3·77	15·0	47·0
33	3·86	2·76	45·5	61·2
37·5	1·65	0·75	79·5	89·5
42·0	1·14	0·19	84·0	97·5
44·5	0·54	0·03	92·5	99·5
47·5	0·26	0·00	96·5	100·0
50·0	0·07	0·00	99·0	100·0

In the case of albumin (Table V) similar results were obtained. Thus, in the unheated liquids at 44 % of saturation with ammonium sulphate about 6 % of the total albumin was converted from the emulsoid to the suspensoid state: at 50 % of saturation with the electrolyte the conversion was of the order of 10 %; beyond this point the precipitation of albumin gradually increased, the precipitation being complete at about 75 % of saturation. In the heated solutions the precipitation of albumin at concentrations of ammonium sulphate greater than 40 % of saturation was considerably increased. Thus, at 42 % of saturation the precipitation was of the order of 29 % of the total albumin; at 44 % of saturation it increased to 33, while at 50 % of saturation the precipitation of albumin rose to the order of 52 %.

E. THE PRACTICAL APPLICATION OF THE ABOVE RESULTS TO THE ROUTINE CONCENTRATION OF ANTITOXIC SERA.

While recognising that the values recorded in Tables IV and V for the percentage precipitation of the individual proteins from their respective solutions may be somewhat higher than when they are associated together in serum, it seemed justifiable to conclude that, in the routine work, by lowering the concentration of ammonium sulphate for the precipitation of the Second Fraction from 50 to 44 % of saturation, there would still be complete precipitation of the pseudoglobulin and antitoxin while the proportion of albumin precipitated at this stage would be appreciably lessened.

TABLE V. *Showing the extent to which the precipitability of albumin by ammonium sulphate is affected by the prolonged heating of its solution at* 57·5° *for* 6 *hours.*

[Percentage of albumin in the original solution = 6·27.]

P_H of the solution = 8·24.

Percentage of saturation with ammonium sulphate	Residual percentage of albumin in solution in the filtrates from the ammonium-sulphate-albumin mixtures		Percentage precipitation of albumin by the ammonium sulphate in the	
	(*a*) before heating	(*b*) after heating	(*a*) unheated liquids	(*b*) heated liquids
20	6·27	6·27	0	0
25	6·27	6·27	0	0
28	6·05	6·14	3	2
30	6·14	6·02	2	4
33	6·05	5·83	3	7·1
37·5	5·95	5·61	5	10·6
42	5·92	4·46	5·5	29·0
44·5	5·84	4·20	6·7	32·9
47·5	5·77	3·86	8·0	38·4
50·0	5·64	2·98	10·0	52·5
56·5	4·76	1·10	23·7	82·5
62·5	3·45	0·81	45·0	87·0
66·5	0·73	0·15	88·5	97·5
70·0	0·18	0·00	97·0	100·0

In order to test this point concentrations were carried out with antidiphtheritic and with antitetanic sera. In both cases a denaturation of the serum proteins to the extent of about 30 % was induced during the initial heating of the serum. Aliquot parts of the filtrates from the First Fraction precipitates were respectively brought up to 44, 47 and 50 % of saturation with ammonium sulphate and the ensuing precipitates were filtered, pressed and dialysed in the usual way.

TABLE VI. *Showing the increase in the degree of concentration of antitoxic sera induced by lowering the precipitation limit for the Second Fraction precipitate.*

Precipitation limits for the Second Fraction precipitates (percentage of saturation with ammonium sulphate)	Percentage of the total antitoxic units appearing in the Second Fraction precipitates	Percentage removal of the total proteins of the original serum	Increased potency per cc.	Units of antitoxin per g. of protein in the final product
Antitetanic plasma*				
30–44	95	69	10 times	6,250
30–50	95	64	8 ,,	5,250
Antidiphtheritic plasma*				
30–44	92	72·5	9·5 ,	18,750
30–47	96	67	8·0 ,,	16,500
30–50	96	66	7·5 ,,	15,000

* Comparable results were obtained irrespective of whether the heat denaturation had been induced by the prolonged heating of serum at 57·5° or by the shortened heating process after the addition of cresylic acid to the serum.

A comparison was then made between the removal of the serum proteins, the increased potency and the relative recovery of antitoxin in the several experiments (Table VI).

From these results it is obvious that for the complete precipitation of the antitoxin and the pseudoglobulin from sera in which a heat denaturation of about 30 % has been induced, the concentration of ammonium sulphate need not be greater than 44 % of saturation.

The adoption of 44 % of saturation with ammonium sulphate in preference to 50 % hitherto recommended presents the following advantages:

(1) The precipitation of heat denaturated albumin with the Second Fraction precipitate is appreciably lessened, thereby ensuring in the end product:

(*a*) a further reduction in the percentage of the total proteins of the original serum without transference of antitoxin to the First Fraction precipitate, and

(*b*) a corresponding increase in the degree of concentration.

(2) The colour of the end products is much less pronounced than when the precipitation limit of 50 % of saturation is used. The extra amount of albumin precipitated between 44 and 50 % of saturation adsorbs an appreciable amount of the colouring matter of serum.

(3) The smaller volume of saturated solution of ammonium sulphate thus required for the precipitation of the Second Fraction precipitate entails a slight reduction of labour and expense.

From the above observations it is clear that, if the precipitation limits for the Second Fraction precipitate be altered so as to exclude the precipitation of heat denaturated albumin, the maximum load of antitoxin which can be carried by one gram of protein in the final product will be somewhat higher than the values previously found [Homer, 1917, 2]. Experimental work in this connexion is in progress and will be published later.

SUMMARY.

During the course of the investigation it has been shown that:

1. The heat denaturation of the serum proteins in alkaline sera is a function of the duration of the time of heating. The maximum effect is produced after four hours' heating at 57·5°.

2. In acid sera the change is complete within one hour's heating at the specified temperature.

3. The heat denaturation of pseudoglobulin and of albumin is a function of the [H·] of their solutions.

4. There is a marked similarity between the curves for the conversion of soluble into insoluble protein during the heating of serum, of solutions of albumin, and of pseudoglobulin throughout the ranges P_H 4·6 to 8·0.

5. The curves for the increased precipitability of the serum proteins and of pseudoglobulin by 30 % of saturation with ammonium sulphate and for the increased precipitability of albumin by 50 % of saturation with ammonium sulphate show a minimum amount of change through the region P_H 5·5 to 8·0 while on the acid side of P_H 5·5 there is absolute coincidence of the curves.

6. In heat denaturated sera the protein precipitated between 30 and 50 % of saturation with ammonium sulphate contains a mixture of pseudoglobulin, antitoxin and heat denaturated albumin.

7. The precipitation of pseudoglobulin from solutions of which the reaction, previous to the liquids being heated, had been adjusted approximately to P_H 8·0 was complete between 42 and 44 % of saturation with ammonium sulphate.

8. The precipitation of albumin from solutions of which the reaction, previous to the liquids being heated, had been adjusted approximately to P_H 8·0 gradually rose from 29 to 52 % between 42 and 50 % of saturation with ammonium sulphate.

9. The practical application of the above observations to the routine concentration of sera has shown us that:

(*a*) The preliminary prolonged heating of sera of which the reaction is on the alkaline side of P_H 7·0 must be for not less than 4 hours and not more than 5 hours at 57·5°.

(*b*) In methods involving a heat denaturation of the serum proteins, the amount of heat denaturated albumin appearing in the final product can be appreciably lessened by including in the Second Fraction precipitate the protein precipitated between 30 and 44 % of saturation with ammonium sulphate instead of between 30 and 50 % of saturation as hitherto employed.

(*c*) This decreased precipitation of albumin entails the production of end products showing a less pronounced colour, a greater removal of the total proteins of the original serum and a degree of concentration considerably greater than if the higher precipitation limit had been used.

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IMPROVEMENTS IN THE TECHNIQUE OF THE CONCENTRATION OF ANTITOXIC SERA.

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The study of the factors influencing the heat-denaturation of serum proteins has led us to change the technique of my method (1916) for concentrating antitoxic sera. These changes involve a considerable shortening of the process and therefore a brief description of our present method of procedure may be useful to those engaged in serum concentration in other laboratories.

Our technique is as follows:

(*a*) *The preliminary treatment of the plasma.*

In order to ensure the desired ease of filtration at the various stages of the process of concentration, the pooled batches of *undiluted* oxalated or citrated plasma are subjected to one of the following alternative methods of treatment previous to their being heated.

1. (*a*) To the plasma is added a sufficient volume of ammonia to adjust the reaction to the value $p_H + 8{\cdot}0$. This may be accomplished as follows:

To 10 c.c. of the plasma is added the necessary volume (x c.c.) of a standard solution of ammonia to bring the hydrogen ion concentration [H] to the value $p_H + 7{\cdot}6$ using neutral-red as the indicator and Sörensen's solutions as standards. To another 10 c.c. of the plasma is added the necessary volume (y c.c.) of the same solution of ammonia to bring the [H] to the value $p_H + 8{\cdot}5$ (faint green tinge with three drops of a 0·1 per cent. solution of α naptholphthalein).

By the addition of $\frac{x+y}{2}$ c.c. of the ammonia solution to every 10 c.c. of the plasma the reaction is approximately adjusted to the value $p_H + 8{\cdot}0$.

(*b*) If the operator cannot conveniently adjust the reaction, then to the plasma can be added not less than 0·25 and not more than 0·30 per cent. of cresylic acid or trikresol.

2. Two per cent. of solid sodium chloride is next added to the plasma treated as above.

(*b*) *The heating of the plasma.*

The heating is conducted in two stages as follows:

Stage 1. Into each of several jars of 22 litre capacity there are measured 14 litres of plasma. The jars are covered and placed in the heating tank containing water at 62°—64° C. whose temperature is maintained between these limits until that of the plasma has reached 57·5° C. The water in the tank is then allowed to cool to 57·5°—58° C., at which temperature it is maintained by means of a thermostat. The tank is covered with a closely fitting lid and *the heating is continued for a further period of four hours.*

Stage 2. At the expiration of the stated period for Stage 1, six litres of a saturated solution of ammonium sulphate are added to each of the jars. The ammonium sulphate content in each jar is thus brought up to 30 per cent. of saturation.

The temperature of the water in the tank is raised to 62°—63° C. and is kept between these limits until the temperature of the plasma-ammonium sulphate mixtures has reached 58° C. Throughout this operation it is essential to keep the mixtures thoroughly well stirred.

As soon as the required temperature has been reached, the jars are removed from the tank and the contents are allowed to cool to 45° C. at which temperature they are filtered. The precipitate thus separated constitutes the First Fraction precipitate.

(*c*) *The precipitation of the Second Fraction.*

The First Fraction precipitates, consisting of euglobulin, heat denaturated protein and a certain amount of pseudoglobulin, are washed with a solution of ammonium sulphate (30 per cent. of saturation), the volume of the latter being about one-half that of the plasma taken for concentration.

The washings from the First Fraction precipitates are filtered and added to the main bulk of the filtrates to which is then added the necessary volume of a saturated solution of ammonium sulphate *to bring the ammonium sulphate content up to 46 per cent. of saturation.*

The ensuing precipitate is filtered, pressed and dialysed in the usual way until free from sulphate. To the residue from dialysis is added 0·85 per cent. of sodium chloride and 0·35 per cent. of cresylic acid. This final product is stored in bulk in the cold room until required.

(d) *The recovery of antitoxin from the First Fraction Precipitates.*

During many years practical experience with the concentration of antitoxic sera in these laboratories it has been found that, no matter how carefully any given method be followed, there has always been a loss of antitoxin, the losses under ordinary circumstances ranging from 5 to 30 per cent. (average *ca.* 18 per cent.).

It has been ascertained that the "missing" antitoxin is not destroyed during the heating of the serum and of the serum mixtures at the temperatures employed, but that it is carried down with heat-denaturated pseudoglobulin in the First Fraction precipitates. The antitoxin is associated with the denaturated protein in such a manner that it cannot be recovered therefrom by extraction with ammonium sulphate (30 per cent. of saturation). However, it has been found that this "missing" antitoxin can be almost completely recovered by prolonged extraction of the First Fraction precipitates with brine in which the heat denaturated protein is soluble to some extent.

In order to reduce our losses to a minimum we have therefore reverted to the Banzhaf-Gibson plan of extracting the First Fraction precipitates with brine, which was long employed in this laboratory. At the same time the method was adopted of dealing with the First Fraction precipitates from a series of concentrations collectively and, since Dr MacConkey[1] had demonstrated that antitoxic sera saturated with salt could be kept at room temperature for months without any appreciable deterioration, his plan of allowing them to soak in brine until they could be conveniently treated, was employed, labour and expense being thereby saved.

Our procedure is as follows:

The First Fraction precipitates after having been washed with a solution of ammonium sulphate (30 per cent. of saturation), are placed in a coarse canvas bag. The bags are dumped into tubs containing a saturated solution of common salt with an excess of solid salt.

Into the same tub are successively dumped the similar precipitates from a series of weekly routine concentrations, the First Fraction precipitates from the concentration of 500 to 600 litres of plasma being usually thrown into about 180 litres of brine. The tubs are kept covered and allowed to stand at room temperature until time can be spared to deal with the liquids. The brined extract of the precipitates is then filtered and to the filtrate is added 0·30 per cent. of glacial acetic acid. The ensuing precipitate is filtered, pressed and dialysed after 3 per cent. of finely ground washing soda has been added to the contents of each parchment dialysing bag. Care must be taken not to overpress the precipitates as in this eventuality great difficulty is experienced in dealing with the residues from dialysis.

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[1] Unpublished observations.

The dialysis is continued until the contents of the dialysing bags are freed from salt. To the residues from dialysis are added 0·85 per cent. of solid sodium chloride and 0·35 per cent. of cresylic acid. The final product is stored in the bulk in the cold room until required.

The above technique presents the following practical advantages over that originally suggested by me (1916):

(1) The preliminary adjustment of the plasma obviates difficulties otherwise so often experienced in the filtration of the hot plasma ammonium sulphate mixtures and also of the final products.

(2) The shortening of the heating in Stage 1 makes it possible to complete the two stages of the heating process within 7 hours.

This is advantageous from the practical standpoint. In a working day of 8 hours' length, the operator can not only carry out the heating processes involved but can also leave the filtration of the First Fraction proceeding overnight.

(3) The precipitation of the Second Fraction by 46 instead of 50 per cent. of saturation with ammonium sulphate considerably reduces the amount of heat-denaturated albumin appearing in the final product. This is advantageous in two ways, for, not only is the degree of concentration enhanced but the colour of the final products is less pronounced.

(4) At a relatively small cost of labour, time and materials the total losses of antitoxin experienced during the concentration of sera can be reduced to a negligible quantity.

Thus, during a series of routine concentrations involving nearly 1500 litres of plasma, the loss with each individual concentration was 15—20 per cent. The subsequent treatment of the First Fraction precipitates with brine resulted in the recovery of an amount of antitoxin which reduced the total loss to 2·5 per cent. These results, based on animal tests, indicate that there had been practically no loss of antitoxin during the process of concentration.

Our present losses are considerably smaller than those experienced with the Banzhaf-Gibson method in which, in order to ensure clear and readily filterable end products, it has been found necessary to throw down the First Fraction precipitates with 33 or even with 36 per cent. of saturation with ammonium sulphate instead of with 30 per cent. as originally advocated.

Recent experiment has shown that, with the higher concentrations of ammonium sulphate thus adopted, the precipitated heat denaturated pseudoglobulin and attendant antitoxin are so influenced that their solubility in brine is considerably decreased. Under these conditions extractions of the First Fraction precipitates with brine does not reduce the loss to the minimum experienced by us in our present procedure.

The similar treatment of the First Fraction precipitates obtained in the Banzhaf (1913) method and in my modification of the latter likewise reduces the loss to a minimum.

In conclusion I desire to express my thanks to Mr Albert Riggs, Head Laboratory Assistant, for his co-operation and for the many valuable suggestions made by him during the working out of the details of the technique described above.

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OBSERVATIONS ON THE PATHOLOGY OF BARCOO ROT (VELD SORE?);

WITH SUGGESTIONS AS TO TREATMENT.

BY

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BY the middle of the summer of 1916 a number of men of the Anzac Mounted Division, which had been operating in the desert east of the Suez Canal for upwards of six months, suffered from spreading superficial ulcers on the back of the hands, extensor surface of the forearms, and, to a less extent, on the back of the feet and on the shins. The ulceration resembles that met with amongst bushmen in the back-blocks of Australia, and is known locally as barcoo rot. It was recognized by the men as the same disease they had seen or experienced in Australia.

Superficial ulcers were also troublesome during the Boer war. They affected troops kept on trek on the high veld, and the disease was labelled "veld sore." A short description of veld sore and its pathology was given by Ogston (1901). Two years later Bishop Harman (1903–4) published a detailed study of veld sore, and I gather from his description that veld sore and barcoo rot are closely allied, if not identical diseases. According to Harman the disease affected Boers and British alike whilst campaigning, but natives were free from it. The Boers did not suffer whilst dwelling on their farms. Harman (loc. cit., pp. 8 and 9) is satisfied from the epidemiological data that the restricted range in diet during operations on the veld is an essential factor in bringing about the condition.

Dolbey (1901) concludes from his experience that troops were not liable to veld sore until they have been at least three months in the country. Life during the dry weather in the Australian back-blocks, and campaigning on the high veld or the Sinai desert, present conditions which are not unlike. In all three cases water for washing is minimal; prolonged subsistence upon bread, biscuit, and salt or tinned meat, with lack of fresh vegetables, is the rule. Further, appropriate dressing of the sores in the early stages is difficult or impossible whilst the patient continues his occupation. Like veld sore, barcoo rot is difficult to cure in the above conditions, but readily yields to treatment when the patient comes into one of the towns. To what extent this is to be attributed to facilities for greater cleanliness, appropriate surgical dressing, or to dietetic improvements, is uncertain.

Brief Description of Barcoo Rot.

The patient may remember abrading the skin, but whether or not, the first sign is usually a bleb or blister containing thin watery pus and surrounded by an area of slightly inflamed skin. The blister may be the size of a sixpence, or even larger, but before it reaches this size it is generally broken. The denuded surface shows little tendency to heal, but slowly extends at its margins, at the same time deepening until there are no more epithelial structures left. The ulcer, however, remains shallow, and it is unusual for the fibrous layer of the skin to be destroyed unless it becomes infected with the virulent staphylococci. The glands are not inflamed unless secondary infection occurs, and there are no constitutional symptoms.

When healing has occurred areas of glossy skin, more or less denuded of hairs and sweat glands, are left. Cicatrization does not occur unless the true skin is destroyed. Infection of fresh areas of skin in the neighbourhood is usual, so that the patient has several ulcers of different ages. The disease in its minor forms is extremely annoying and, when the ulcers have become large, incapacitating.

The symptoms and pathological findings have no resemblance to the slowly evolving leishmaniasis known under the names of *Bouton du Nil*, *Clou de Biskra*, *Bouton du Caire*, etc.

Observations on the Pathology of Barcoo Rot.

On a visit to an outlying field ambulance I was shown twenty men admitted for obstinate ulcers of considerable extent. The ulcers were shallow, mostly on the back of the hands and wrists—that is, in areas exposed to small injuries, and rich in hairs. The smooth surfaces were free from ulcers, and the progress of an ulcer appeared to be inhibited as soon as it extended to a hairless portion of skin.

It seemed advisable to examine the state of the hair follicles, both within and around the ulcers, and I plucked a number both from the base of the ulcer and from the reddened slightly swollen skin at the margin; many of the hairs were loose. I wrapped the hairs in paper moistened with 70 per cent. glycerine for transport. On my return to the laboratory the hairs were stained by Gram's method and counterstained very slightly with weak fuchsin, dehydrated, and examined in clove oil or balsam. In all of the hairs withdrawn from the base of the ulcers, and in many from the inflamed margin, Gram-positive microbes were present in the hair sheath. They were mostly cocci, but in the hairs from three of the cases bacilli, which at the time I took to be diphtheroids, were also present. The infection extended usually to the lower end of the hair follicle.

Some of these cases were subsequently removed to No. 3 Australian General Hospital, Cairo, for observation and study. They were placed under the care of my comrade Captain Maclure, to whom I am much indebted for his kind co-operation.

Infection of the hair follicles was confirmed by microscopical examination of fresh material, and cultures from the bases of removed hairs and from the contents of early blebs were made upon blood agar and ordinary agar. *Staphylococcus albus* was the predominating organism, but some *S. citreus* and a few *S. aureus* were also present. In one case a diphtheroid was abundant. This proved to be *B. diphtheriae*,* but I did not discover this until some time afterwards. Diphtheroids are not uncommon in lesions of the skin, such as ulcers, vaccinia pustules, impetigo, and eczema,† and I regarded it as a chance concomitant of the staphylococcal infection, and did not investigate it further at the time. The case differed in no obvious way from the others, and there were no constitutional symptoms at the time.

The microbial infection of the hair follicles appeared to be confined to the connective tissue sheaths, except in very badly affected hairs, in which the base was disorganized and the layers of the root sheaths unrecognizable.

The pus from the early blisters before they were broken afforded only growths of staphylococci. I generally found there was a hair, often a very small one, passing through them, and the follicle of the hair was invariably infected with staphylococci.

The cocci cultivated were, as mentioned above, mostly varieties of *Staphylococcus albus*, with a few *S. aureus* and *S. citreus*. Their virulence is presumably low, for in uncomplicated cases there is no tendency to invade the true skin, and no involvement of the lymph glands occurs.

A few experiments were made upon the biochemical characters and pathogenicity of some of the cocci isolated. Six white staphylococci colonies, derived from four patients, were examined as to their action on milk and gelatine and their fermentation of lactose and mannite. They all clotted milk and liquefied gelatine with varying rapidity and fermented lactose. None fermented mannite. They were not haemolytic for human blood as tested by Eijkmann's agar plate method. They appear, therefore, to correspond to Welch's *Staphylococcus epidermidi albus*, as defined by Gordon (1906).

The cultures of *Staphylococcus aureus*, and two of *S. citreus* from hair follicles which were tested in the same way, fermented mannite. I could not make a number of experiments on animals, but two rabbits injected intravenously with 2 c.cm. of a broth culture of the white cocci and one with the same quantity of the broth culture from the *aureus* colonies were not made manifestly ill.

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* Fatal for guinea-pigs in doses of 0.5 c.cm. eighteen hours' broth culture in thirty hours; 0.1 c.cm. antitoxin protected against 2.5 c.cm.

† The records of the finding of diphtheroid bacilli in skin lesions are collected by Graham Smith in Chapter IX of his book in collaboration with Nuttall, *The Bacteriology of Diphtheria*, Cambridge University Press, 1908.

My observations on the bacteriology of barcoo rot are in general accord with those of Ogston (1901) and Harman (1904) upon veld sore. Ogston and Harman, however, make no mention of any infection of the hair follicles. Ogston saw only Gram-positive cocci in fluid from the edge of ulcers. Harman cultivated only staphylococci from the unbroken vesicles. These gave the cultural characters of *Staphylococcus pyogenes aureus*, but grew rather more abundantly on culture media than a virulent strain with which they were compared. They were not virulent for rabbits. Harman was much impressed by the resistance to heat, to the effects of drying, and to the action of corrosive sublimate possessed by his cultures, but this was not, I think, greater than has been met with in some strains of staphylococci.

The epidemiology of veld sore was carefully studied by Harman, but he could find no indication that the infection spread from one to another among the troops, and barcoo rot affects men living in solitude. Neither disease seems to be associated with particular occupations. It would seem probable that barcoo rot, and possibly veld sore too, is due to the infection of the epidermal structures with staphylococci of low virulence, such as abound upon the skin. In barcoo rot the infection of hair follicles is apparently the common way in which it begins, and the usual manner in which it spreads at the margin of the ulcer.

Harman's view of the pathology of veld sore I take to be that under ordinary nutritive conditions the skin cocci of low virulence are not able to "make good" against the local defences, but that in the case of men too long upon a restricted diet the defence is weakened, and they are able to infect the epidermal structures but not the true skin. He regards it, in fact, as a minor scorbutic manifestation. This may well be, but improper dressing and neglect of the sores also play an important part, for I have seen them heal up rapidly under appropriate dressings when no improvement in diet was possible. The fact recorded by Harman that the African natives did not suffer whereas the Boers did suggests, however, that lack of washing and of simple surgical dressing is alone an inadequate explanation.

Treatment of Barcoo Rot.

In hospital the ulcers generally heal up slowly under any sort of wet antiseptic dressing, but are very difficult to deal with in the field. A wet dressing is unhandy when a man has to go about his business and it is difficult or impossible to change it sufficiently frequently. Moreover, in a hot climate it soon becomes converted into a dry dressing and adheres to the ulcer.

Harman found that if the ulcers were thoroughly cleaned up, powdered with calomel and covered with cotton-wool, the last being affixed by strapping, good results followed. This method is satisfactory for small ulcers, but the cleaning up must be very efficiently performed.

In view of the observations on the pathology of barcoo rot described above, it seemed not unlikely that the infection of the hair follicles was largely responsible for the continuance of the ulcerative process in the centre, and its progress at the margins of the ulcers. An infected hair follicle is like a foreign body, and healing will not occur until it is either sterilized or ulcerated out. Acting upon this idea I removed the hair or hairs traversing the early blebs, and found that these healed straight away. The patients soon realized the efficacy of early removal of the hair follicles in suppressing these secondary infections, and undertook this part of the treatment themselves. Next, working on the principle employed to restrict a bush fire by burning an area in front of it, I attacked the larger ulcers, removing the hairs from their base and around their margins, and was pleased to find that the ulcerative process was stayed, and healing commenced at once.

The extensive ulcers in the floors of which no epithelial structures remain, naturally take a long time to heal, but this period Captain Maclure abbreviated by the transplantation of numerous snippets of skin from the abdomen.

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TYPES OF DYSENTERY BACILLI ISOLATED AT No. 3 AUSTRALIAN GENERAL HOSPITAL, CAIRO, MARCH—AUGUST, 1916, WITH OBSERVATIONS ON THE VARIABILITY OF THE MANNITE FERMENTING GROUP.

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and

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The results of attempts to isolate Dysentery bacilli from 217 cases in which the stools contained muco-pus with or without blood are tabulated below. In many cases the amount of mucus in the stool was very small. All these cases occurred amongst Australian troops of the E.E.F.

TABLE I.

Amoeba histolytica or cysts present		63
B. dysenteriae Shiga isolated		47
Mannite fermenting dysentery bacilli	Agglutinated at time of isolation by Y serum*	64
	Not agglutinated at time of isolation by Y serum	12
No causative organism recovered		36
	Total	222

* Serum made from Hiss and Russell's Y bacillus at the Lister Institute, original titre 1/8000.

In five instances dysentery bacilli were isolated from patients with amoebic infections.

The method employed was to wash the mucus, break it up in sterile broth and plate off some drops on the surface of a MacConkey plate. Next day likely colonies were picked off, sown into warm broth and incubated for a few hours. They were examined for motility and if motile discarded. The non-motile broth cultures were sown into glucose, lactose and mannite peptone water and on to an agar slope. If glucose or both glucose and mannite were fermented with the formation of acid only and lactose unchanged an emulsion was made from the agar slope and tested as regards agglutinability against a Shiga or Y serum respectively. Macroscopical methods were employed.

In forty-nine instances the further biochemical characteristics of the strains were investigated immediately.

The Shiga types of dysentery bacilli isolated were all true to type and agglutinated as a rule well at a dilution of 1/800 with a serum made

TABLE II.

Variation in the Fermentations, etc., of organisms agglutinated by Flexner-Y Serum.

	Maltose	Saccharose	Dextrin	Raffinose	Arabinose	Isodulcite	Sorbite	Glycerin	Indol
1	A	O	A	A	O	O	O	O	+ +
2	O	O	A^4	A	A^6	O	O	O	+ +
3	A	O	O	A	O	O	O	O	O
4	O	O	O	A	A^4	O	O	O	O
5	O	O	O	O	A^4	O	A	O	+ + +
6	O	O	O	A	A^6	O	O	O	O
7	A	O	O	O	O	O	A	O	+ + +
8	A	O	O	O	A^4	O	A	O	+ + +
9	O	O	O	A	O	O	O	O	+ +
10	O	A^7	O	A	O	O	O	O	O
11	O	O	O	O	O	O	O	O	O
12	O	O	O	A	O	O	A	O	+
13	O	O	O	A	O	O	O	O	O
14	A	A^6	A	A	O	O	O	O	+
15	O	O	O	A	O	O	O	O	+
16	O	O	O	A	O	O	O	O	+
17	O	O	O	A	O	O	O	O	+
18	O	O	—	O	—	O	A	O	+ + +
19	A	O	—	O	—	O	A	O	+ + +
20	A	O	—	O	—	O	A	O	O
21	A	O	—	O	—	A^3	O	O	O
22	A	O	—	A	—	O	O	O	+
23	O	O	—	O	—	O	O	O	O
24	A	O	—	O	—	O	O	O	+
25	O	O	—	A^5	—	O	A	O	O
26	A	O	—	O	—	O	O	O	+ + +
27	O	O	—	O	—	O	A	A^9	+ +
28	A	O	—	O	—	O	O	O	O
29	O	O	—	O	—	O	O	O	O
30	A	O	—	A	—	O	O	O	+
31	O	O	—	O	—	—	A	O	+
32	O	O	—	O	—	—	A	O	+
33	O	O	—	A	—	—	O	O	+
34	O	O	—	A	—	—	O	O	O
35	O	A^7	—	A	—	—	O	O	O
36	O	O	—	O	—	—	O	O	O
37	O	O	—	A	—	—	O	O	+

at the Lister Institute. Higher dilutions were not tried as the serum, although specific, had not a high titre for the homologous organism at the time we were using it.

The organisms of the mannite fermenting group were, on the contrary, very variable, both as regards their biochemical activities and agglutinability. Forty-nine of them were tested upon a variety of carbohydrates. They all failed to form acid in lactose, dulcite, inulin and adonite. They all produced acid in glucose, mannite and galactose and turned milk first acid then alkaline. Their action upon maltose, saccharose, dextrin, raffinose, arabinose, isodulcite (rhamnose), sorbite and glycerin and their power to form indol varied with different strains and the same strain varied at different times. The details of these variable reactions are given in Tables II and III. Table II includes all those which at the time of isolation were agglutinated by our Y serum, Table III those which were not.

TABLE III.

Variable Fermentations, etc., of organisms not agglutinated by Y Serum at the time of isolation.

	Maltose	Saccharose	Dextrin	Raffinose	Arabinose	Isodulcite	Sorbite	Glycerin	Indol	Agglut. by Patient's serum	Agglut. by polyvalent curative serum	Agglut. by Y serum 6 months after isolation
38	A	O	—	A	—	O	O	A^3	+	1/400	1/250	O
39	A	O	—	A	—	A	O	A^3	+	O	—	1/1600
40	A	O	—	O	—	—	O	O	O	O	—	—
41	A	O	—	O	—	A	O	O	+	—	—	—
42	A	O	—	O	—	—	O	O	+	—	—	—
43	O	O	—	O	—	O	O	A^5	O	O	O	O
44	O	O	—	O	—	—	O	O	O	—	—	—
45	A	O	—	O	—	A	O	O	O	1/200	—	1/800
46	A	O	—	O	—	A	O	A^9	O	1/800	—	1/400
47	O	O	—	O	—	O	O	O	+	O	—	1/1600
48	A	O	O		A	A	A	A^4	O	1/400	1/250	O
49	O	O	—	O	—	O	A	O	+	—	O	O

A = Acid only in peptone water containing the various carbohydrates in 24 hrs.
A^3 = ,, ,, ,, ,, ,, ,, ,, ,, 3 days.
O = Growth but no change in reaction.
— = Not tried.
+ = Indol formation determined by paradimethylaminobenzaldehyde and for + + and + + + strong reaction of indol with above reagent.

Thirty of the cultures in Table II were kindly re-examined for us by Miss Rhodes of the Lister Institute, about six months after isolation. Meantime they had been subcultured at intervals on agar slopes. Out of the three originally fermenting saccharose two had lost this property.

Five had lost the power to split maltose and other five had acquired it; four had lost the power to split sorbite, and the other two had acquired it. In six cases the action upon raffinose was reversed, three ceasing to ferment this sugar and three gaining this faculty.

The proportion of indol was the most variable; the property being lost in nine cases and acquired in four.

The instability in biochemical activities manifested by these Egyptian strains, other than in the action upon lactose, dulcite, glucose and mannite, is in accordance with observations upon organisms of this group isolated in different parts of the world, and a survey of the voluminous literature on this subject convinces us that any attempt to separate the mannite fermenting dysentery bacilli into groups on the ground of their action upon carbohydrates is unsound.

Fermentation of Saccharose.

Strong (1900) isolated a bacillus from cases of dysentery in the Philippines which differed from Flexner's organism derived from the same source in not fermenting maltose but attacking saccharose. On these grounds it has been regarded as a new species of dysentery bacillus but we understand from Prof. Strong that he is not of this opinion.

The inference that saccharine fermentation differentiates Strong's from Flexner's bacillus was disturbed when Hiss (1904) found that at this time two out of three strains of the latter fermented saccharose in peptone water more readily than the former, twelve days elapsing in the case of Strong's bacillus before the development of acidity.

There are two strains of Strong's bacillus at the Lister Institute which have been propagated for several years. When one of us had occasion to examine these two strains a few years ago they would neither of them attack saccharose. We notice also that Fraser (1916) says that a culture of Strong's bacillus obtained by him from the Director of the Bureau of Science, Manila, failed to ferment saccharose. The capacity to split cane sugar is obviously a variable characteristic, with a tendency to disappear upon laboratory media. Three of our cultures fermented saccharose at the time they were isolated and two retained this power after six months. The property may more quickly disappear. Hehewerth (1916) found that three out of twenty-six strains isolated during a small epidemic fermented saccharose but all lost this faculty four months later. Fraser (1916), too, gives instances to the same effect, in which the faculty had disappeared in fourteen days.

So far we have been principally concerned with the disappearance of the power to split cane sugar. By artificial selection strains possessing this characteristic may also be derived. Twort (1907) propagated a Flexner's bacillus, which at the time did not ferment saccharose, upon relays of peptone water containing this sugar, subculturing about every fourteen days. After some months the strain was found to possess the characteristic of readily fermenting saccharose.

The same faculty may arise spontaneously. In 1915 it was noticed that the strains of Flexner at the Lister Institute were inconstant as regards the fermentation of saccharose and maltose in peptone water and at the same time displayed a tendency to form secondary colonies[1].

Variations in carbohydrate fermentation were studied in detail by Massini (1907) and Burk (1908) and Kowalenko (1910) with Neisser's (1906) *Bacterium coli mutabile*. Lactose fermentation is, however, the stable character of this organism, acid-forming colonies breeding true and non-acid-forming colonies breeding both kinds. It seemed more likely that the nature of the character of saccharose fermentation would, in the case of dysentery, more closely resemble the isodulcite fermentation of typhoid and dysentery bacilli investigated by Müller (1908, 1911) and by Penfold (1911), and the lactose fermentation of typhoid bacilli by Penfold (1911). In these latter cases the faculty to ferment the sugar is the variation from type. The white colonies on Endo or MacConkey's media breed true and the red colonies mixtures, and on subculture on to media devoid of the particular sugar the faculty becomes undiscoverable after a few subcultures.

In the case of dysentery bacilli, however, even when propagated on broth media, the fermentation of saccharose may again, for reasons unknown, become a characteristic of the strain of sufficient prominence to be observable.

We investigated one of our strains which at the time of isolation produced acid in peptone water after a few days. Our object was to determine whether the faculty could be enhanced by selection and whether it possessed any stability. We were unfortunate in our choice for of the three strains originally fermenting saccharose this particular one soon lost this power, whereas the others have retained it during six months.

To increase the number of microbes in the culture capable of fermenting the sugar we used the method of Neisser (1906).

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[1] Personal communication by Dr Harriette Chick.

17. vi. 16.	Sown in saccharose peptone water.
24. vi. 16.	Acid reaction developed.
27. vi. 16.	The 10th day, plated on neutral red saccharose agar.
28. vi. 16.	50 pink colonies; 800 colourless colonies which remained so five days.
	Subcultures from colourless colonies bred only colourless colonies, which remained colourless.
	Two pink colonies sown in saccharose peptone water.
3. vii. 16.	One of the peptone waters developed acid in five days, other not.
	From former a plate of saccharose neutral red agar made.
4. vii. 16.	300 colonies.
6. vii. 16.	All pink.
7. vii. 16.	18 *colonies have developed daughter colonies of bright red colour.*
	Red bud sown in peptone water.
8. vii. 16.	Peptone water strongly acid. Loopful planted on saccharose agar.
9. vii. 16.	350 colonies developed.
10. vii. 16.	All colonies pink; 50 developing buds.
11. vii. 16.	Buds well grown and bright red. Bud sown in saccharose peptone water and on to another saccharose plate.
12. vii. 16.	Peptone water acid in 16 hours. About 800 colonies on plate all pink.
14. vii. 16.	45 colonies developed red buds.
15. vii. 16.	Material from a bud plated on to saccharose agar.
18. vii. 16.	Numerous colonies developed red buds but the colonies were too thick for enumeration.
	One bud sown in saccharose peptone water.
19. vii. 16.	Peptone water acid in 15 hours.
	At this stage the process of artificial selection was suspended as we were apparently not increasing the character. We had long since ceased to get any colourless colonies. All colonies turned pink, but the proportion developing secondary colonies remained about the same.
20. vii. 16.	Material from a bud was sown into broth and subcultured every few days for one month.
20. viii. 16.	Broth culture sown in saccharose peptone water and plated on saccharose neutral red agar.
21. viii. 16 to 31. viii. 16	No acid formed in 10 days in the peptone water. Only permanently colourless colonies developed upon the saccharose plate.

In this strain the power to ferment cane sugar was a feeble characteristic, tending to disappear and only developed and maintained by natural or artificial selection.

During the course of this experiment an interesting and unusual change in the agglutinability of the organism occurred. On the 4. vii. 16 it was agglutinated completely by a dilution of 1 in 800 of our Y serum. On the 9. vii. 16, that is, after we had selected from daughter colonies, it was retested and was agglutinated equally well, but on the 21. VII. 16 it was not agglutinated by a dilution of 1 in 50. This was repeated and the serum we were using tested and there was no apparent source of error. The non-agglutinable culture was kept and propagated upon nutrient agar, being subcultured at fortnightly intervals. A few months later it was retested and found to agglutinate as well as ever.

As far as we are aware, variations, whether natural or induced, in the fermentative characters of bacilli are not usually accompanied by serious alterations in their agglutinability. Kowalenko (1910, p. 289). Penfold (1911, p. 51).

Jacobsen (1910) has, however, recorded an instance of daughter colonies possessing different agglutinability to the mother colonies in the case of his *Bacterium typhii mutabile* which was isolated from cases of typhoid fever in a lunatic asylum. The mother colonies of this organism were inagglutinable by typhoid serum, whereas the daughter colonies were agglutinated in a dilution of 1 in 10,000. Further, by propagating from single bacilli of the mother colonies in broth, agglutinability gradually developed until in four months the full sensitiveness was reached.

Fermentation of Maltose.

Maltose and saccharose fermentation were properties utilized by Lentz (1913) to differentiate mannite fermenting dysentery bacilli into groups. To what extent this is likely to be useful in the case of saccharose has been just discussed. The fermentation of maltose is a considerably less stable property of these dysentery bacilli than that of saccharose. Hiss (1904) showed that his Y bacillus fermented maltose after successive cultures on media containing this sugar. In our observation one-third of the strains examined had in six months changed in this respect. Hehewerth (1916) examined his cultures at intervals up to four months after isolation and found that in twelve out of twenty-six the action was reversed, some acquiring the faculty to ferment maltose, others losing it. Individual colonies from the same patient behaved differently.

Other Carbohydrates. With the remaining carbohydrates, sorbite, raffinose, isodulcite and dextrin, the action is uncertain and varies from time to time. Indol production is equally capricious.

The action of the organisms isolated in Egypt upon isodulcite demands a short comment. It will be noticed that but one in Table II fermented this sugar and that late, whereas five out of the twelve which were not, at the time isolated, agglutinated by Y serum, did so.

Isodulcite is only exceptionally fermented by any of the Flexner or Y strains, but, as shown by Müller (1908), by growing the former upon agar containing this pentose, daughter colonies may be formed which readily attack it. On the other hand Morgan (1911) found that a strain of Strong's bacillus *B. pseudo-dysenteriae* D Kruse and some of Ruffer and Willmore's (1909) El Tor strains produced acid in isodulcite with varying rapidity.

Morgan also examined the cultural characteristics of a number of strains of dysentery-like organisms fermenting mannite which Ledingham had isolated in the course of an extensive investigation upon typhoid carriers in Great Britain. In no case was there any reason to suppose that the individuals from whom they were derived were suffering or had suffered from dysentery. The majority of these strains fermented isodulcite and about half of them were agglutinated by Y serum in high dilution.

We come to the conclusion therefore that for purposes of isolation and identification of the dysentery bacilli, the only carbohydrates of service are glucose, mannite, lactose and dulcite.

The Agglutination of the Mannite fermenting dysentery bacilli.

Hiss (1904) divided these bacilli into three groups, Y, Strong and Flexner, by serological reaction. He showed that a serum made with his Y bacillus agglutinated Flexner's bacillus nearly as well as the homologous serum. On the other hand, a serum made with Flexner's bacillus did not agglutinate the Y bacillus in high dilution. The agglutinins for the Flexner bacillus in a serum made from Y bacillus were completely absorbed by an emulsion of Flexner's bacillus, leaving those for Y intact. Reciprocally Y bacilli only absorb the agglutinins for Y out of a Flexner serum, leaving the titre for the Flexner bacillus undiminished.

Kruse (1907) found at least six groupings necessary to accommodate the strains he examined, the sixth being a cave of Adullam for those which would not comfortably fit into one or other of the first five groups. The majority of organisms isolated from epidemics in Germany, up to this time, fell into either his Group A or D. Kruse suggested that his Group D is the same as that represented in America by the Y bacillus of Hiss and Russell, but had not a culture of the latter for comparison.

Morgan (1911), however, found that although Y serum agglutinated *B. pseudo-dysenteriae* A Kruse and one specimen of *B. pseudo-dysenteriae* D Kruse to the full titre of 1/20,000 of the serum and another specimen to 1/500 only, in no case did these three bacilli remove the agglutinin for Y. *B. pseudo-dysenteriae* D Kruse must therefore be regarded as serologically distinct from Y.

The agglutinations of Egyptian "El Tor" strains of dysentery were examined by Ruffer and Willmore (1909). From experiments with an El Tor serum, a Flexner serum and a *B. pseudo-dysenteriae* D serum, Ruffer and Willmore placed the mannite fermenting dysentery bacilli into two groups. The first contains El Tor No. 1 and *B. pseudo-dysenteriae* D Kruse, and in the second they place *B. dysenteriae* Flexner and *B. pseudo-dysenteriae* A Kruse. The members of each group could, however, be differentiated by absorption experiments.

Later, Morgan (1911) studied El Tor bacilli, *two years after their isolation*, along with other well-known strains, and found that the former as well as the latter were all agglutinated in a dilution of 1/2000 of a serum he prepared from a specimen of Hiss and Russell's Y bacillus and, with one exception, by a Flexner serum but in a much lower dilution.

Winter (1912) also examined thirty-one strains of this type of dysentery bacillus; thirty were derived from German sources and one was a Flexner bacillus obtained from America. He found Castellani's absorption method unsatisfactory and confined his observations to cross agglutination experiments. On this basis eleven of his strains appear to correspond to Kruse's *B. pseudo-dysenteriae* D and seven to Kruse's A Group. Three are so poorly agglutinated by any of his sera that it is uncertain how they should be classed, but they appear to belong to Group A. Two are intermediate between Groups A and D, and the remaining twelve cannot be grouped by the sera employed.

Hutt (1913) emphasizes the futility of grouping dysentery bacilli according to their action upon carbohydrates. This had previously been pointed out by Kruse. He used the absorption method and thereby confirmed Kruse's groupings, indeed increasing their number.

The position may be summed up by saying that the mannite fermenting dysentery bacilli comprise a number of strains serologically distinguishable by the absorption method, but overlapping considerably as regards agglutination. For the diagnosis of an organism suspected to belong to this dysentery group no one serum is adequate: sera made with a member of each of Kruse's groups A, D and E, would embrace most of the German strains. Y serum seems to be least specific and covers the greatest range.

From Morgan's work Y serum seemed to be particularly indicated for diagnosis work in Egypt, and we used it exclusively. Nevertheless, nearly one-sixth of the bacilli we recovered in 1916 were not agglutinated by Y serum directly after isolation. The titre of our serum against the homologous organism was 1/4000 at the time of using.

Eight of these strains were kindly re-examined by Miss Rhodes six months later. Four of them were then agglutinated by the same Y serum in dilutions, varying from 1/400 to 1/1600, so that in the meantime they had acquired some sensitivity by cultivation. Two of the four strains which still failed to be agglutinated were clumped by the polyvalent curative serum of the Lister Institute[1] in a dilution of 1/250.

In eight of the cases we were able to test the organism isolated against the patient's own serum and in four of them it was agglutinated in dilutions above 1/100 (see Table III), indicating that he had been infected by the microbe recovered from his stool. Two of the strains which did not acquire agglutinability on culture are included amongst those which were agglutinated by the patient's serum.

It would be wrong to assume that a bacillus with the morphological, cultural and biochemical characters of the Flexner group of dysentery bacilli is discredited as an etiological factor because it is not agglutinated by Y or any other univalent serum. This group of organisms is clearly in an unstable condition and records frequently occur of strains which are not agglutinated by sera prepared from the common type-strains. A recent instance, which is interesting on account of the unusual toxicity of the strain for rabbits, is given by d'Herelle (1916). This organism, which possessed the usual characteristics of the Flexner group, was isolated in five cases during a small epidemic amongst a troop of Dragoons and was not agglutinated by the polyvalent Flexner Y serum of the Pasteur Institute.

Summary and Conclusions.

1. Of 123 dysentery bacilli isolated, forty-seven were *B. dysenteriae* Shiga, seventy-six were mannite fermenters.

2. The biochemical activities of forty-nine strains of the latter were investigated immediately after isolation and again six months later.

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[1] In the process of immunization the horses furnishing this serum had been injected with a number of Egyptian strains received from Sir Armand Ruffer some years ago.

They showed variability as regards the fermentation of maltose, saccharose, dextrin, raffinose, arabinose, isodulcite, sorbite and glycerin and in the formation of indol. The same strain varied at different times, some gaining, others losing one or other of the above properties.

Similar observations by other observers and the authors are discussed and the conclusion is arrived at that the separation of the mannite fermenting dysentery bacilli into groups on the ground of their action upon the above carbohydrates is unsound and that the only carbohydrates of service for their identification are glucose, mannite, lactose and dulcite.

3. An experiment conducted during two and a half months upon a particular strain of dysentery bacillus shows that the fermentation of saccharose was in this strain a "recessive character" and only maintained by artificial selection.

4. One-sixth of the mannite fermenting dysentery organisms isolated were not agglutinated by a univalent Y (rabbit's) serum at the time of isolation but half of these acquired this property by cultivation. Others were well agglutinated by the patient's serum. An experiment is described in which agglutinability to Y serum was lost under prolonged cultivation on saccharose peptone media. In this experiment successive cultures were made every few days and the material for subcultivation was taken from daughter colonies (buds), which arose in the saccharose plates. Agglutinability was rapidly regained by propagation in broth. From the author's experience and from a survey of the literature, the conclusion is arrived at that no one univalent serum will agglutinate all dysentery bacilli of the mannite fermenting type and that a bacillus with the morphological, cultural, and biochemical characters of dysentery bacilli of this type is not discredited as an etiological factor because it is not agglutinated by any particular univalent serum.

In conclusion we express our indebtedness to Miss Rhodes of the Lister Institute for retesting many of the cultures in London, in January, 1917, and convey to her our grateful appreciation of her kindness in so doing.

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Recent Researches into the Ætiology of Typhus.

By MURIEL ROBERTSON, M.A.

(From the Lister Institute of Preventive Medicine.)

"TYPHUS fever is an infectious disease which runs an acute course of from twelve to fifteen days and culminates spontaneously in a more or less abrupt lysis. It is characterized by an incubation period of from five to twenty days, a high continued pyrexia and a petechial rash."

This definition of the disease is quoted from Wilder, 1911; he adds that the method of dissemination "is of a peculiar nature not dependent upon the direct transmission of the contagium from the sick to the well, but rather by the transfer of body parasites (lice) carrying the contagium."

In opening this discussion I propose only to touch quite superficially and in passing upon the historical aspect of the question, then to consider the more probable views as to the nature of the actual virus, and finally to give a short critical account of the experimental evidence relating to the transmission of the disease by insects, notably by pediculi.

Typhus is a disease of dirt, misery, crowding and famine, it is associated with periods of unrest and political upheaval and above all with war. In the individual fear and mental anxiety are frequently noted as adjuvant circumstances. The disease has a wide distribution but is restricted to temperate climates. When it appears in warmer regions it is only to be found at high altitudes or during the cold season. Thus in Mexico typhus occurs on the high cool plateau and not on the tropical seaboard; similarly in North Africa and in India it is a winter and a mountain disease.

From the historical point of view typhus is not particularly interesting, largely because it has not got any very dramatic symptoms by which it can be recognized in early and imperfect accounts. Ancient and mediaeval writers were in a happy state of confusion about all fevers, and typhus seems to have appeared along with plague, relapsing fever, malaria and typhoid, with all of which it has at various times been confused. It was first recognized by Girolamo Frascatori in 1505 as a fever distinct from plague, and the name "petechie" was used for it. Nevertheless it was long considered to be only another and less severe manifestation of the very familiar and much dreaded pest. The confusion of *typhus exanthematicus* with typhoid fever occurs all through the literature up to within quite recent times, and in Germany there is to this day a certain inclination to consider them as one.[1]

From the beginning of the sixteenth century onwards there is a succession of typhus epidemics in every country in Europe; wars and bad harvests, either alone or operating together, seem to bring this disease in their train as an almost inevitable concomitant. There is no particular interest in following these dismal records, it is more to the point to note the very few instances in which wars did not produce serious outbreaks of typhus. As far as I have been able to discover France and America are the only countries where a war has not inevitably brought this disease.

France has rather a curious history in regard to typhus in the nineteenth century: from the end of the Napoleonic wars up to 1852 there is a complete absence of typhus. The winter of 1852-53 was very cold and food was scarce in Paris; dysentery, bronchitis and pneumonia became frequent and in the early spring of 1853 typhus broke out. Interestingly enough this epidemic was not restricted to the poor and badly housed alone, but affected all classes; the mortality in the private practices among the wealthier and therefore better nourished people was, however, very low, only 1 per cent., whereas among the hospital patients drawn from the poorer classes the mortality was 21 per cent. This seems to point to the important connexion between the severity of the disease and the state of nutrition of the population. The army returning from the Crimea brought typhus again into France and there were several epidemics, which, however, died down in the course of the next few years. Since then, except for small outbreaks occurring in Normandy and Brittany, involving insignificant numbers, France has been singularly free from typhus. Even the Franco-Prussian war did not, according to Chauffard, produce an epidemic. This may perhaps have been due to the short duration of the campaign.

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[1] *See* for example O. Mueller, *Med. Klin.*, 1915, as quoted in the *Trop. Dis. Bull.*, viii, No. 1, p. 57.

The only other considerable war which remained free from typhus was the American Civil War.

In the present war typhus has not so far appeared among the Allied armies on the Western Front, but Russia, Serbia, Macedonia and Germany have, as is well known, all had epidemics.

The mortality in different epidemics of typhus varies enormously: it may be as low as 9·9 per cent., as in an outbreak in Dublin in 1836-38. An epidemic with an even lower mortality—namely, 2·5 to 4 per cent.—is noted by Haeser as having taken place in Scotland in 1843. Caldwell, 1916, notes that in the recent epidemic in the Balkans the hospital mortality ranged between 19 and 65 per cent. In 1846-48 there were outbreaks of typhus in Flanders, and Haeser states that in the Canton of Mons the mortality was only 1 per cent., whereas in another district of Flanders there were 734 cases, of which 693 died, equalling a mortality of 94·4 per cent.

One feature stands out very distinctly, especially in the later and more clearly differentiated epidemics, and that is the close connexion of famine with typhus; in every case a scarcity of food seems to create the particular conditions that foster this disease. No such connexion is found to anything like the same extent for instance in epidemics of plague or cholera or small-pox. Curiously enough European relapsing fever seems also connected more particularly with periods of hunger.

Before leaving this aspect of the question, which will I hope be treated in detail by some of the epidemiologists present, I would like to touch upon an account of typhus in Serbia given by Dr. Jeanneret-Minkine. This is a vivid and interesting little book, though in some respects it is superficial and not very accurate. It is, nevertheless, a convincing description recorded at first hand by an experienced and observant medical man. The author is much impressed by the effect of the psychological factors of fear and depression in predisposing to the disease, and as affording a bad prognosis when infected. He also notes the fatigue and exhaustion of the soldiery at the time of the outbreak of the epidemic. In many instances the hospitals themselves were active centres for the actual spread of the contagion, and he gives a number of striking instances to show that pediculi were accountable for the spread of the disease. He notes for instance that the only hospital where typhus did not break out was an English one at Uskub, which was able to keep itself free from lice, and where the well instructed personnel refused to take patients except in the numbers that their bathing and receiving arrangements could cope with. Jeanneret-Minkine describes his own hospital as being simply flooded with patients who could not be washed, for whom they did not have clean shirts, and for whom there were no blankets; they had therefore to lie in their uniforms. All the medical men took typhus except one, who assured his colleagues that he had been able to keep free from lice.

The author thinks that cimex may also transmit the disease, but the evidence he adduces is not sufficiently sound, and the louse channel is not excluded in the instance cited. He notes among his clinical observations the great frequency of secondary infections, such as abscesses of the parotid gland, a certain number of septicæmias, and not infrequently pneumonia. I wish to draw attention to these complications in typhus as they are interesting in view of some of the many organisms obtained by various observers from the blood of typhus patients. And in this connexion I would like to note how certain epidemics as a whole seem to be marked by a frequent appearance of a particular complication—for instance, the great tendency to gangrene of the feet in the Serbian epidemic. In a bad epidemic in Silesia in 1847 bronchial catarrh and pneumonia were very prevalent complications. These bronchial features are also noted in an Irish epidemic cited by Haeser. Jeanneret-Minkine lays stress in the Serbian epidemic on the well-known feature of the bad condition of the mouth, and considers that many of the secondary infections have obtained their entry by this means. I think that in some of these features we have the explanation of the many cocci, &c., described from the blood of typhus patients, to some of which I must refer in a later part of this account. Jeanneret-Minkine's observations upon the experimental side of our knowledge need not delay our attention.

In passing to the consideration of the actual nature of the virus it is interesting to notice the rapid advance in knowledge that has occurred since 1909, when Nicolle first succeeded in producing experimental typhus in monkeys. Once the investigator can remove a disease from the region of mere description and what one may call police court evidence, into the sterner atmosphere of experiment and scientific deduction, the mystery begins to yield, and although the typhus problem is not yet by any means solved, we have nevertheless a good deal of knowledge about it. The literature is vast and often contradictory, and I can only claim to have done my best to condense the results of the more important work into the brief space of this discussion. It will be easier to give an epitome of typhus ten years hence than it is to-day, as authors are still at that stirring stage in research when they contradict each other cheerfully upon matters of fact. The earnest disciple, therefore, can have a number of the basic facts relating to typhus according to two or three conflicting doctrines. It is sometimes possible for the critic to arrive at a conclusion from the evidence put forward, but there are many instances in which that is not feasible.

It was clear when Nicolle was able to produce typhus reaction in monkeys by the injection of blood from a patient suffering from the disease that the virus was present in the peripheral blood. Since then monkeys have been used, and of late years also guinea-pigs, as experimental animals in typhus research, and a great deal of our knowledge has been derived from the study and the control of the reaction of these animals under various conditions of experiment. It is rather important, therefore, to consider at once the value of these animal tests. In the case of the monkey I am myself convinced that there is no doubt whatever that the injection of virulent typhus blood produces a marked and clearly defined febrile reaction. It is, however, of the utmost importance that a careful and prolonged observation of the individual animal in regard to its normal thermal chart be made before the test injection. Small variations in temperature are without significance, and many monkeys in captivity run high and irregular temperatures due to causes outside the experiment. These factors must of course be taken into account. One other point can also be disposed of—namely, that monkeys do not in my experience, nor in that of a number of workers, show any febrile reaction to the mere injection of human blood into the peritoneum. I have no personal experience of the typhus reaction in guinea-pigs, but it appears that they also are useful animals though not so susceptible as monkeys; here also the charts require to be considered strictly with respect to the normal chart of the individual animal.

The experimental animal having been found and the whereabouts of the virus determined, the next problem was, in what part of the blood did the infective agent reside, and what was its nature? With this end in view Nicolle, Conor and Conseil carried out experiments in Tunis, and further work was also done by Ricketts and Wilder in America, and also by Anderson and Goldberger.

Nicolle, Conor and Conseil, in 1911, came to the conclusion that the virus was contained in the leucocytes. They considered the plasma, which they found less infective, owed its virulence only to the leucocytes or leucocytic débris from which it is difficult to free the plasma. They further state that the centrifuged blood-serum obtained after clotting is not infective, a result which Anderson and Goldberger do not confirm. Nicolle and his colleagues also give an experiment in which the washed red corpuscles failed to infect, although the blood as a whole was quite virulent. They tested the cerebrospinal fluid, which was clear, and found it devoid of virulence. Anderson and Goldberger repeated Nicolle's experiment, separating the blood elements with great care, and come to conclusions in which they criticize some of the French workers' interpretations. I quote the summary from Anderson and Goldberger, which seems upon the evidence brought forward to be a sound series of deductions (1912, p. 61).

> "(1) That the evidence adduced by Nicolle, Conor and Conseil does not especially favour their hypothesis of an intraleucocytic localization of the virus of typhus—on the contrary the infectivity of the centrifugated blood serum obtained after clotting with its low leucocyte content would be in favour of a parasite free in the circulating plasma of the blood; (2) that the blood serum of virulent typhus blood is constantly infective, whether obtained from defibrinated blood or after clotting, instances of its apparent avirulence being explicable by a natural resistance of the monkey; (3) that it may perhaps be possible to deprive typhus blood serum (obtained after clotting) of its virulence by prolonged centrifugation, but that this does not necessarily indicate an intraleucocytic localization of the virus; and (4) that repeated washings of the blood corpuscles do not deprive them of their infectivity, a fact explicable by the physical phenomena involved in centrifugation."

In connexion with this question of the localization of the virus in the blood, Anderson and Goldberger carried out a pretty experiment with citrated blood from the heart of a guinea-pig infected with anthrax. They centrifuged the citrated blood and made plates from measured quantities of the separated elements. The distribution works out as follows: "Whole blood," 100 organisms; "centrifuged plasma," one organism; leucocyte cream, 10,000 organisms; "reds," ten organisms. In this case where there is an organism free in the plasma, its relative distribution in the different blood elements agrees exactly with the relative infectivity put forward by Nicolle and his colleagues for the same blood elements in virulent typhus blood.

Valuable work done by Ricketts and Wilder, and independently confirmed by Anderson and Goldberger, shows that the virus is contained in the serum obtained by centrifuging the defibrinated blood and also that filtration through a Berkefeld candle robs the serum of its infectivity. This experiment, carried out by Ricketts and Wilder in February, 1910, is carefully documented. The serum was divided; one part inoculated without filtration into a monkey produced a typical attack of typhus; the filtered portion produced no effect whatever. This animal is subsequently tested for susceptibility and shows a perfectly definite typhus reaction. Wilder, in addition, repeated his experiment with the following result: the monkey which received the unfiltered serum showed a typhus reaction, while the animal which received the filtered material remained quite unaffected. When, however, both monkeys were subjected to an immunity test at a later period both were found to resist the attack; the control monkeys to this experiment showed the usual typhus infection. The resistance of the monkey which had received the filtered serum can be explained by one or more hypotheses which the author states, without however selecting the one he prefers.

(1) The monkey may have been naturally immune.

(2) The animal may have been immunized by the filtered serum.

In this connexion Wilder points out that there are variable factors in filtration experiments: for instance he used a different candle, though of the same make and number; also the suction pressure was not controlled. Therefore in the second experiment products may have come through which were excluded in the first instance. In addition the two experiments were made from the blood of different patients who were not at the same stage of the disease. Wilder also suggests that the organism may be pleomorphic and may differ in size at different stages. And he finally adds that fragments of the organism or toxins may have come through which were capable of immunizing the animal.

Nicolle, Conor and Conseil, in their paper of October, 1910, have also one experiment in which a monkey which had received filtered serum from clotted typhus blood showed itself immune to a subsequent infection of virulent typhus blood. This animal (No. 47) exhibited a small rise of temperature, of however less than 1° C., between the fifteenth and twenty-first day after receiving the filtered serum. The French workers look upon this as a positive experiment. In several other attempts the filtered serum gave uniformly negative results. It must be noted that Wilder in all his work is not sufficiently on his guard against the important factor of a natural immunity against typhus either temporary or permanent in a monkey, and Nicolle and his colleagues do not consider this possibility at all. It is to the credit of Anderson and Goldberger that they did become alive to this very cogent feature, and their experiments reveal a tolerably frequent occurrence of animals who showed a natural immunity against the disease. They state that "22·5 per cent. of rhesus monkeys possess at least a transient natural immunity, and it seems reasonable to consider that in about 3·5 per cent. of monkeys the resistance noted amounts to a permanent natural immunity."

The temporarily immune monkey may receive a full dose of infected blood without showing any reaction. They are however not immunized by this, and will react at a second or third inoculation given subsequently. Anderson and Goldberger, who carried out several filtration experiments, point out that Nicolle's so-called filter-passing experiment hinges entirely on this question of the possible natural immunity of the monkey. The American authors have themselves an experiment in which a monkey received a dose of filtered serum to which he did not react; after thirty-four days he received an inoculation of 6 c.c. of virulent blood and showed no febrile reaction. The authors point out that at this stage they might have concluded that the filtrate had immunized this monkey. However they went on and gave another injection of virulent blood in seventy days from the injection of the filtrate and the animal developed a very typical typhus fever. They have another experiment also exactly parallel to this one.

To summarize these points: There is no experiment recorded, as far as I am aware, in which a monkey showed a typical typhus reaction upon the injection of filtered serum from a typhus patient. There are about twelve experiments in which filtered serum produced no febrile reaction and in which the monkey was not immunized by the injection of the filtered serum. The experiments recorded in which filtered serum from typhus patients appeared to immunize a monkey against a subsequent infection, do not exclude the possibility of the monkey being naturally immune. I conclude, therefore, that the evidence indicates that the virus of typhus will not pass a sound Berkefeld filter.

All the authorities are agreed that one attack of typhus in an experimental animal confers a solid immunity against subsequent attacks.

So much then for the work on the virus treated as a whole, carried out by workers who considered that they could not see it nor cultivate it. I have now to consider some of the work of the people who thought they could either see the infective agent, or grow it, or both. I cannot consider anything like all the views put forward along these lines: I have selected representative examples.

Plotz, in 1915, isolated a pleomorphic bacillus from cases of typhus in New York. He describes his method—the organism is an anaërobe. He isolated the same organism from eight guinea-pigs and one monkey, which were experimentally infected with the virus of typhus obtained from Anderson and Goldberger. Plotz and his colleagues Olitzky and Baehr used their particular method of cultivation of the blood in a number of other febrile conditions such as typhoid fever, tuberculosis, acute osteomyelitis, acute rheumatic endocarditis, &c. They state that "in none of these 198 cases was an organism recovered which in any way resembled the bacillus isolated from cases of typhus fever."

One of these workers, Olitzky, carried out a detailed series of serological tests which I cannot deal with here, and comes to the conclusion that he is dealing with specific agglutinins and precipitins. In the next section of this paper the three authors record experimental work with the virus derived from cases of typhus, or from animal passage, and with the cultures isolated from cases. The animals used were guinea-pigs. They cultivated the pleomorphic anaërobe from a certain proportion of animals inoculated with the typhus virus. To quote their results in their own words: "Of nine guinea-pigs with mild febrile reactions, in only one was the blood-culture positive. On the other hand, in fifteen animals with severe reaction, the blood culture was positive in seven, or almost 50 per cent. This relation between the blood cultures and the severity of the disease was also observed in the human typhus cases."

The weakest part of this work lies in the fact that the cultures isolated proved to be incapable of producing the disease in a quite convincing manner in animals susceptible to typhus. Two cases only are cited where a reaction was produced upon injecting the culture into guinea-pigs, and neither can be held to be perfectly conclusive. Subsequent experiments were absolutely negative, which the authors explain along the usual lines, that the subculture on artificial media robs the organism of its virulence.

Plotz's work has now been confirmed by Popoff, who investigated an epidemic of typhus in Macedonia; the results were published in the *Deutsche Medizinische Wochenschrift*, in April, 1916. He repeated Plotz's work, using precisely the same method, and obtained an organism which tallied with that described by the American authors. Blood cultures were most likely to be successful if made before the crisis and at the height of the rash. He then proceeded to test the sera of typhus patients for the presence of agglutinins, and found that he could obtain a very clear reaction with sera taken from the patient at or after the crisis. Agglutinins were not present as a rule in the earliest days of the disease, but developed from the crisis onwards and showed the highest titre about seven to fifteen days after the crisis. Popoff could find agglutinins in the sera of two men who had had typhus ten months previously. Moreover he found that the sera of two men, vaccinated three and four months previously with Plotz's bacillus, agglutinated his strains isolated in Macedonia, thus suggesting that the organism isolated by Plotz in America and by the author in Macedonia were identical in this respect.

Popoff put up satisfactory controls to his experiments and his paper reads convincingly. It is true that it may be objected that the demonstration of an organism in the blood during the height of a disease, and the subsequent development of agglutinins specific to that organism, are not in themselves proof that the said organism is the exciting cause of the disease. Nevertheless in many instances—for example, that of typhoid fever—such evidence would be taken as authorizing a diagnosis which nobody would dispute. It can very reasonably be held that *Bacillus typhi-exanthematici* of Plotz is a secondary invader, but it must be admitted that the evidence is beginning to accumulate in favour of this organism. I have given Plotz's work in full, as in conjunction with that of Popoff it seems to constitute the best documented case of any of the organisms grown from typhus blood. Typhus has a bad reputation and pathologists and bacteriologists have a certain impatience with any new claimant in the way of an organism said to be the exciting cause of this disease. If typhus had a clean slate and started as a new condition about which we had no views, Plotz's work would be treated much more seriously and Popoff's confirmation would add the weight of an independent repetition of the result. It may also be noted that a minute organism free in the plasma would fit in with the experiments of Anderson and Goldberger as to the seat of the virus in the infected blood. Plotz obtained further confirmation from Olitzky, Denzer and Husk, who repeated his work in Mexico with similar results. There are some interesting points in this paper, but I cannot go further into them at present. Plotz's bacillus was grown from the presumably infected lice.

I must now consider some of the other organisms put forward as playing a part in typhus.

Rabinowitsch has consistently grown a cocco-bacillus by aërobic methods from the blood of typhus patients—he considers it to be the causal agent of the disease. I think, as in so much of the work along these lines, that he puts up a very good case for the presence of the organism, but the causal connexion between it and the disease does not appear to me to be established.

Wilson grew cocci from the blood of typhus patients in Ireland, but considered them to be secondary invaders.

Penfold obtained a hæmophilic diplococcus also from cases in Ireland, and I have myself confirmed this result, using his method. One point however came out, that out of the two cases the first gave very few colonies, but the blood was quite virulent when injected into six monkeys, the incubation period being six days; the second patient was past the crisis, and the monkeys failed to react to the injection during nineteen days, when the observations were given up owing to circumstances beyond my control—i.e., the Sinn Fein rebellion—but the blood cultures gave many more colonies than in the case of the first patient. This is, I think, evidence on the side of the coccus being a secondary invader.

Hort and Ingram, in a brief note in 1914, claimed that the exciting cause of typhus fever was a pleomorphic aërobic bacillus which they described somewhat comprehensively to be the same as Plotz's organism and as that described by Rabinowitsch. This seems scarcely probable as the cultural characters seem somewhat divergent.

Hegler and Prowazek (1913) carried out some investigations into typhus. Prowazek noted, in fifty-one cases examined, alterations in the polymorphonuclear neutrophil leucocytes. These changes were not seen in cases of measles and amœbic dysentery, &c., investigated at the same time. He observed fragmentation of the nucleus and in addition round or oval bodies which are sometimes double; these bodies are well-defined, with sharp contours, and stain an intense carmine colour with Giemsa's method. The bodies are never internuclear. Prowazek distinguishes these appearances from Doehle's bodies. The bodies appeared in the leucocytes about the third day of the disease; they persisted after defervescence and were present in one case as late as the nineteenth day of the illness. Monkeys infected with typhus showed these bodies in their leucocytes; they were not found in infected guinea-pigs.

We now come to deal with the transmission, and here again the pioneer work was done by Nicolle and his colleagues in Tunis. In 1909 these workers obtained the first transmission of typhus from monkey to monkey by means of body lice. The details of the experiments are as follows: The virus was obtained from a patient on May 19, on the third day of his illness and at the time of the appearance of the rash, and inoculated into a chimpanzee. After an incubation of twenty-four days this animal showed a febrile reaction corresponding in type with that of typhus. Blood from this chimpanzee was inoculated into a *Macacus sinicus*, which developed a typical attack of typhus. Body lice, twenty-nine in number, were fed on this monkey on the sixteenth day after inoculation. The next day, and for the following days until the lice had all died, they were allowed to feed on two bonnet monkeys, A and B. Monkey A was bitten by lice varying in number from fifteen to three. Monkey B was bitten for fourteen days by lice varying from fourteen to three in number. The lice were kept between the feeds at a temperature of 16° to 20° C. Monkey A showed no change for twenty-two days when there was a rise in temperature. The fever remitted slowly from the thirtieth day to the thirty-fourth; there was a second attack of fever on the thirty-ninth day, and the monkey died on the forty-fourth day. While this is not a perfectly regular typhus chart it must be noted that fevers of this type may be produced by the direct inoculation of the virus into monkeys. Monkey B had a long incubation period—namely, forty days—during which the chart of its temperature ran in a perfectly normal manner; there was a febrile reaction from the forty-first to the forty-sixth day, and the temperature declined by crisis. The passages of blood made from monkeys A and B on to other monkeys did not give rise to any definite typhus reaction, and the authors call them "réactions febriles avortées." They attribute their lack of success to the lowering of the virulence by passage.

Ricketts and Wilder in 1910 made experiments similar to those of Nicolle and found that they could immunize monkeys against infection with virulent blood by the bites of infected lice. In a later paper Wilder (1911) gives the results of further work upon this question. Eight monkeys were subjected to the bites of lice previously infected by feedings on human typhus patients. Five of these were positive and the monkeys were immunized by this exposure to the bites of the insects, although to quote the author's own words: "In none of our insect experiments were we able to provoke a very characteristic febrile reaction, although slight fever was observed in nearly every case, but when a monkey is exposed to the bites of infected lice he is thereby immunized to typhus fever, so that he proves refractive when later injected with virulent blood."

Wilder carried out rather an important experiment as follows: Six lice were allowed to feed three times at intervals of twenty-four hours on a typhus patient during the eighth, ninth and tenth day of his illness; the lice were then kept for forty-eight hours at a temperature of 11° C. to 12° C. without food. The intestinal contents of these six lice were introduced into scarifications of the skin of monkey No. 32. The animal showed no reaction, but he had developed a solid immunity and failed to react to the injection of 4 c.c. of virulent blood; the control monkeys reacted quite typically. The interesting features in this case are that it shows that the lice became infected some time between the second and fifth day after feeding; it is also pretty clear that the causal agent must have multiplied in the lice in that time as the quantity of blood contained in the insects during the experiment cannot have exceeded 0·06 c.c., which is much below the lowest infecting dose of the most infectious blood. The minimum infecting dose lies in direct passage of typhus blood between 0·2 c.c. and 1 c.c. of defibrinated blood. It must, however, be remembered in considering the results of this experiment that Wilder was not sufficiently alive to the possibility of natural immunity in the monkey, and one would like to see this piece of work repeated.

Wilder made an interesting investigation by rearing the eggs deposited by infected lice. The young lice were never themselves directly exposed to infection. These young lice were allowed to feed upon a normal monkey (No. 42), which was subsequently tested for immunity. Wilder considers that this monkey was rendered immune, but the chart he gives on p. 89, which shows the temperature of the monkey after receiving the virulent blood which constituted the immunity test, is not quite satisfactory, as there is a slight febrile reaction on the ninth day. Wilder considers that this reaction is so much slighter than that of the controls that he concludes that the monkey was immune, and "owed his immunity to his previous infection by the young lice, and that hereditary transmission of the infectivity of the louse is established to the extent of reasonable probability." Looking at this result of Wilder's with the eye of criticism, one cannot help considering that the evidence, though suggestive, is rather slender to establish so important a point. It should be noted that Anderson and Goldberger failed to obtain any evidence of hereditary transmission of the virus in the louse; their experiments with lice hatched from presumably infected mothers being definitely negative.

Anderson and Goldberger made experiments in the transmission of typhus by lice which corroborate and extend those of Wilder; they conclude that both *Pediculus vestimenti* and *Pediculus capitis* may become infected with typhus, and that the infection may be transmitted by subcutaneous injection of the crushed insects or by their bites. Anderson and Goldberger failed to transmit the virus of typhus by means of the bites or by the subcutaneous injection of crushed bed-bugs.

In considering the value of all these experiments on transmission detailed above, it is clear that the actual experimental demonstration hinges upon the question of the immunity of the monkey. Natural immunity occurs and is a factor which obscures the result, nevertheless the number of experiments is sufficient to render the question virtually settled. One curious feature calls for notice—namely, that as Anderson and Goldberger show very clearly, a full dose of virulent blood if it fails to produce a febrile reaction also fails to immunize. That is to say, that in direct passage of the virulent blood there is no immunization unless there is a febrile reaction. Now in the transmission by lice the monkey is apparently immunized without the production of a febrile reaction, and the blood of such a monkey if injected into another monkey fails to produce infection. This is rather a curious discrepancy which still needs clearing up.

Before concluding I should like to refer to the quite recent work of Sergent, Foley and Vialatte, and also to that of Rocha-Lima. Sergent and his colleagues describe cocco-bacilli from lice from cases of typhus; they note their affinity for Giemsa's stain and a tendency to bipolar staining. They conclude that if these cocco-bacilli do not constitute the virus of typhus themselves one may consider that they are, as is the case with several Pasteurella, microbes which are witnesses that accompany the true and invisible agent of the infection. Rocha-Lima, in 1916, found in 95 per cent. of lice from patients in a prison epidemic in 1914, large numbers of bodies which took on a red colour with Giemsa's stain. He thought they rather resembled bacteria without being absolutely the same in appearance. Prowazek examined these bodies and considered them to be the same as those found by him in the blood of the patients to which reference has been made above. In handling the lice both Prowazek and Rocha-Lima contracted the disease, of which Prowazek died. The shape of these structures varies from short elliptical coccus-like bodies to definite rods. The usual bacterial stains, such as methylene blue, carbol fuchsin, and carbol thionin, fail to colour the bodies satisfactorily; Loeffler's flagellar stain, carbol gentian violet, and in particular Giemsa's solution, stain the bodies very clearly. The author is convinced that these bodies are micro-organisms. Cultivation of the organisms on agar and ascitic agar failed. Rocha-Lima has eight instances in which he produced typhus in guinea-pigs by injection of the contents of lice. In two cases he injected part of the gut contents of a single louse after having assured himself by microscopical examination that the material contained the suspicious bodies. This louse-virus had been kept going for nearly a year by passage from guinea-pig to guinea-pig. At the eleventh passage it was injected into a monkey, which exhibited a typical typhus reaction. This louse-virus immunized against the infection with the virus derived from patients. The author admits that bodies similar to those described may very exceptionally be met with in lice which are not derived from patients infected with typhus.

Rocha-Lima extends his work in a later paper (May, 1916), and cites some interesting experiments. He took lice which were presumably normal and let a certain number of them feed on typhus patients, the rest he fed on normal people under similar conditions. Those fed on the typhus patients developed the bodies described above, the other group did not show them. The author now names these bodies *Rickettsia prowazekei*; he is of the opinion that they belong to the Strongyloplasmata and should be looked upon as Chlamydozoa. The parasites are located in the cells of the gastro-intestinal tract of the lice. Cultural attempts made aërobically and anaërobically upon a large variety of media have so far failed.

When Rocha-Lima kept the lice at 23° C. between the feeds upon the infected patients, the Rickettsia did not develop, and the injection of the material into guinea-pigs did not produce infection. If, however, they were kept at 32° C., the Rickettsia did develop and the injected lice produced infection in guinea-pigs. Rocha-Lima found that the lice were not infective till the fifth day after feeding on infected blood. It will be noted that his results are in direct contradiction in regard to the optimum temperature at which to keep the lice to those of Nicolle, Comte and Conseil, of Ricketts and Wilder, and of Anderson and Goldberger. Rocha-Lima states without protocols that he has had positive results in experiments dealing with hereditary infection in lice. The larvæ hatched from eggs laid by a mother on the sixth day after feeding on typhus-blood have shown themselves to be infected. Noeller tried feeding pig-lice on typhus patients, and at the same time let human lice suck the same patient's blood. The Rickettsia developed in the human lice and not in the pig-lice.

I cannot touch for lack of time upon the application of serum-therapy to typhus; work along these lines has been carried out by Nicolle and his colleagues in North Africa.

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NOTES ON THE OCCURRENCE OF *B. TETANI* IN WOUNDS.

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(Paper read at a Meeting of the Society of Tropical Medicine and Hygiene, Friday, October 19th, 1917.)

The following is an account of some of the results obtained during the course of an investigation into the occurrence of *B. tetani* in the wound exudate of 252 cases which were not shewing any symptoms of tetanus intoxication.

The work was carried out at the suggestion of the War Office Committee for the Investigation of Tetanus, to whom a preliminary report of the main results was made in October, 1916. I am indebted to the Committee for their kind co-operation in arranging facilities for me in regard to obtaining material and in many other details.

The cases were chosen at random from four different hospitals in the London area between the dates of August 17th and September 28th, 1916, the only common factor in the selection being that the wounds were classed by the surgeons and sisters under the general heading of "septic wounds." The cases were all receiving treatment of one kind or another: eusol, hydrogen peroxide, physiological salt solution, hypertonic saline, bismuth-iodoform-paraffin paste, boric fomentations, malachite green, cyanide wool, chlorinated soda, potassium permanganate, iodine, zinc sulphate, glycerine, hypothymaline, hypochlorous acid, eucalyptus, enzymol—all figured, either singly or in various combinations, under the heading of "treatment" in the case-sheets. No attempt was made to neutralise the antiseptics used in the dressings, the samples were dealt with just as taken direct from the wounds. The period elapsing between the date upon which the man was wounded and the date upon which the sample was taken was always noted, and varied from a few days to several months.

The exudate from the wound was collected upon a sterile swab or in a sterile pipette, care being taken to get a representative sample. The material was emulsified in sterile saline and inoculated into culture media. Various media were used, but the main line of the investigation was carried out by using an autoclaved chopped-meat medium, the recipe of which is added at the end of the paper. The emulsified wound material was inoculated into such a meat-tube, and the tube was placed for fifteen minutes in a water-bath at 80° C. This heating gets rid of the non-sporing organisms; it seems also to throw out *B. perfringens* in a proportion of the cases, though not by any means in all. After heating, the meat-tube is cooled in water and placed in the anaerobic cylinder. On certain occasions, when there was an excessive number of tubes to handle and the anaerobic cylinders were overcrowded, another method was adopted. One or two cubic centimetres of sterile liquid paraffin were run into the tube of meat medium, which was then steamed for an hour or longer: after cooling, the material from the wound was inoculated into the tube, which was placed in the bath at 80° C. for fifteen minutes, and thereafter incubated at 37° C. without any further precautions. The paraffin seal to the meat-tubes is not really necessary and may be omitted, but care should always be taken to see that the meat-medium tubes are steamed shortly before use. These aerobic methods are very convenient and are quite satisfactory, especially for mixed cultures; nevertheless, for prolonged work and for further subcultures, an anaerobic apparatus of some kind is desirable.

The meat cultures were incubated for four or more days, and examined microscopically for the presence of anaerobic organisms. In the cases in which these organisms had developed, subcultures were made into broth and egg-broth, which were incubated anaerobically for seven days or longer. These subcultures in broth or egg-broth were used for inoculation into mice and guinea-pigs, and in every case a control animal received an equal dose to which had been added thirty U.S.A. units of tetanus anti-toxin.

The presence of *B. tetani* was held to be demonstrated when—

(1) The characteristic tetanus reaction had been produced by the inoculation of the culture into an animal.

(2) When the control animal had been protected by the addition of thirty U.S.A. units of tetanus antitoxin to the dose of culture injected.

No attempt was made to obtain pure cultures of the organism. *B. tetani* was always found to be accompanied by one or more other anaerobes, and the unravelling of these mixed cultures would have rendered the handling of any sufficient number of cases quite impossible. It will be seen in the sequel that, under the particular conditions obtaining, the exhibition of tetanus toxin was not prevented by the presence of the other anaerobes in the cultures.

The wound exudate from 252 cases of patients shewing no symptoms of tetanus intoxication was examined, and, in addition, the exudate from 45 cases from patients suffering from declared tetanus was also investigated.

I. THE 252 CASES SHEWING NO SYMPTOMS OF TETANUS.

The cases were divided into three classes according to the cultural results :—

(1) Those in which organisms of the "drumstick" type with round endspores were cultivated along with other anaerobes. These amounted to 64 cases, *i.e.*, 25·5 per cent.

(2) Those in which anaerobes of various kinds not including any types shewing round endspores were cultivated. These amounted to 105 cases, *i.e.*, 41·6 per cent.

(3) Those in which there was no growth of anaerobes. These amounted to 83 cases, *i.e.*, 32·9 per cent.

From the above it will be seen that the total percentage of cases in which anaerobes of one kind or another were grown is 67·1.

It was not possible, owing to practical considerations, to carry out a toxicity test with everyone of the 169 anaerobic cultures obtained from the whole series under investigation. The following tests were, however, made :—

Sixty cultures belonging to class 1 (*i.e.*, those cultures shewing round endspores) were tested for toxicity, and in nine a tetanus reaction was produced in the animal injected. That is to say, that tetanus toxin was developed in 15 per cent. of the cases shewing organisms with round endspores. These positive cultures fulfilled the criteria laid down above :—

(1) The characteristic tetanus reaction was produced by the inoculation of the culture into an animal.

(2) The control animal was protected by the addition of 30 U.S.A. units of tetanus antitoxin to the dose of the culture injected.

And, in addition, the cultures had revealed organisms with round endspores.

Forty-four cultures belonging to class 2 (*i.e.*, those cultures which shewed anaerobes other than types with round endspores) were tested for the presence of tetanus toxin. In no case was a tetanus reaction produced in the animal injected.

Taking all the cultures tested, both from class 1 and class 2, together, the figures give 104 cases tested and 9 positive tetanus, *i.e.*, 8·6 per cent. This is a manifestly incorrect way of handling the figures, as in the series of 252 cases the numerical relation of class 1 to class 2 is as 25 to 41, *i.e.*, as 1 to 1·6, whereas in the sample tested the ratio of these classes is 60 to 44, *i.e.*, 1 to 0·7.

A fairer way of putting the question is to consider that the nine positive tetanus reactions were obtained upon a series of 252, which makes the percentage equal 3·5. The chance that the untested residue of class 2 would have yielded further positive tetanus reactions is small, having regard to the result of the tests actually carried out in the two classes.

Table I gives the distribution of the cases according to the length of time elapsing since the date of wounding. The table includes up to the end of the third month. This takes in all except eleven cases where the wound was of still longer standing. It is interesting to note that the cases in which living *B. tetani* were demonstrated fall into two periods, namely, within (*a*) seven days of wounding and (*b*) between the 30th and 60th day of wounding. The period between the 30th and 60th day of wounding presents features of particular interest. (It must be borne in mind that the whole series of 252 are already selected from the bulk of wounded, in that they were chosen as being "septic wounds.") In a total of 91 cases from this period the distribution falls out as follows :—

Cases with round endsporers, *i.e.*, class 1 ...	17,	*i.e.*, 18·7 per cent.
Cases with anaerobes other than round endsporers, *i.e.*, class 2	41,	*i.e.*, 45·1 ,,
Cases where no anaerobes could be cultivated, *i.e.*, class 3	33,	*i.e.*, 36·2 ,,

The cases in which living *B. tetani* were demonstrated amount to 7, *i.e.*, 7·6 per cent. Of the cases falling into this period belonging to class 1 (*i.e.*, those shewing round endsporers) the whole 17 were tested for toxicity and produced 7 positive tetanus reactions, a percentage equal to 41·1. From class 2 (*i.e.*, those shewing anaerobes other than round endsporers) 21 cases were tested for toxicity, and produced no tetanus reaction in any of the animals injected. Now the ratio of those tested in each of classes 1 and 2 from this period is 17 to 21, *i.e.*, 1 to 1·2, which while lower than the ratio of these two classes in the entire series of 252, is nevertheless pretty near it. The ratio over the entire series is 1 to 1·6.

If the cases for the first month after wounding are taken together—they amount to exactly 100—one gets the following percentages:—

33 per cent. of cases with round endsporers.

39 per cent. of cases with anaerobes other than round endsporers.

27 per cent. of cases with no anaerobes, and in this series of 100 there are only 2 per cent. where it could be demonstrated that the patient's wound contained living *B. tetani*.

It therefore appears that, under the conditions of treatment, the second month is a period of greater danger from the point of view of tetanus being present in sufficient numbers to be demonstrable than the first month. All the patients had had a prophylactic injection of tetanus antitoxin immediately upon being wounded.

The actual time elapsing between the date of wounding and the taking of the sample in the nine cases in which tetanus was demonstrated is as follows:—

7 days in case No. 235.
8 ,, ,, 146.
33 ,, ,, 12.
37 ,, ,, 123.
39 ,, ,, 236.

45 days in case No. 73.
49 ,, ,, 27.
56 ,, ,, 62.
56 ,, ,, 68.

Case no 146 had some features of particular interest—the sample was taken upon the eighth day after wounding, the patient having received one prophylactic dose of tetanus antitoxin in France. The meat culture made from the wound exudate gave a very good growth of organisms with round endspores accompanied by a rather slight admixture of another anaerobe of a proteolytic oval-spored type. The subinoculations in broth gave actively toxic cultures producing tetanus symptoms in mice and guinea-pigs, which symptoms failed to appear in the control animals receiving the same dose to which was added thirty units of tetanus antitoxin. That is to say, the wound contained an actively toxic strain of *B. tetani*. The condition of the patient was, however, good, and the wound was reported to be going on well. On the thirtieth day after wounding a second sample was taken, and investigated for the presence of anaerobes without result, the only organisms grown being cocci. The patient made an uninterrupted recovery. In this case the prophylactic dose had apparently tided the patient over without symptoms, although his wound contained a toxin-producing strain of *B. tetani*, and the organism was subsequently dealt with and eliminated during the progress of the healing of the wound.

Cases Nos. 73, 27, 62 and 68 all gave cultures which were actively toxic, although the periods at which the samples were taken were respectively 45, 49, and 56 days after the date of wounding. *B. tetani* was, as usual, present with other anaerobes ; the patients were shewing no symptoms of tetanus intoxication, and the surgeons did not consider that the condition of the wounds gave any cause for anxiety. To have a sufficient number of *B. tetani* organisms present in a wound for cultures to be grown from a swab or from a pipette containing not more than 0·25 to 0·5 of a c.c. of exudate, 56 days after the date of wounding, seems of necessity to imply that the organisms have been multiplying to a certain extent in the wound. The strains were capable of producing toxin as was shewn by the reaction in the inoculated animals. By the fifty-sixth day the effect of the prophylactic dose (which is usually administered within the first 24 hours from the time of wounding) would, according to all the experimental evidence, have disappeared. It would therefore seem, either that the organism in the wound was under conditions unfavourable to the production of toxin, or that the patient had produced antibodies on his own account and was able to deal with any small quantity of toxin that was being absorbed. The evidence at present available leaves the question unsolved. Cases such as these where *B. tetani* is harboured for long periods do of course account for the late cases of tetanus which appear from time to time.

To consider now the significance of organisms with round endspores in cultures from a wound. It is perfectly clear that in itself it is not an indication that the patient is actually harbouring *B. tetani*. This is quite evident from the figures given above, and is further supported by the fact that an organism of this morphology, which differs from *B. tetani* in cultural characters and which is non-toxic, has been isolated from several of the cultures of the above series. This organism is a strict anaerobe: it is motile, it produces gas and a pink colour in meat, does not liquify gelatin, does not liquify inspissated serum, produces acid without clot in milk and ferments maltose and glucose. It is non-toxic and non-pathogenic for guinea-pigs. This organism agrees with one first isolated by Dr. MacIntosh, a culture of which he very kindly placed at my disposal. It has also been mentioned by Adamson and Cutler, and has, I believe, been recognised by many workers dealing with wound material. The fact, however, remains that it is from the group of cases containing the round endsporers that the toxic cultures were obtained; one is, therefore, led to the conclusion that the particular conditions which foster round endsporers as a whole also foster *B. tetani*. This conclusion is supported by a great deal of direct evidence from cultural data which cannot be gone into in the present paper. One thing, however, is strikingly shewn, namely, that the endsporing group comes up late in the mixed cultures in the meat-medium tube, its appearance being preceded by members of the proteolytic group.

This point as to the value of the round endsporer as a warning in regard to the condition of the wound will be touched upon again in dealing with the exudates from the cases of patients suffering from declared tetanus.

I do not propose to deal with the individual organisms in this paper; suffice it to say that a very considerable number of different anaerobes were found to be present, both pathogenic and non-pathogenic. Thus a strain giving all the cultural characters of the "vibrion septique," and very pathogenic for guinea-pigs, was isolated from one of these cases by Miss Hempl, of Ann Arbor University, U.S.A., who was working in the

Lister Institute at the time. Several members of the non-pathogenic proteolytic group were also obtained, so also *B. perfringens* and several oval endsporing organisms.

I would like to take this opportunity to correct an error in nomenclature in a previous paper. I identified the proteolytic organism so frequently found in wounds with the organism described by VON HIBLER as malignant œdema of KOCH. The cultures current in this country under the title of malignant œdema agreed in detail with what I was recovering from the wounds, and agreed with VON HIBLER'S account, except that they were practically non-pathogenic. I am now inclined to think that malignant œdema of KOCH, as described by VON HIBLER, must be an impure culture consisting of a proteolytic sporebearer, and some one or other of the pathogenic forms. In any case I think the name is not a good one and should be given up. The proteolytic form may, I think, be considered to be *B. sporogenes* of METCHNIKOFF, but here again this is in some doubt, as METCHNIKOFF considered that his *B. sporogenes* was closely allied to *B. enteritidis sporogenes* of KLEIN; this is another of the anaerobe types that is not well-defined, and it appears to have characters in KLEIN'S account that resemble *B. perfringens*. The name *B. sporogenes* has, however, been adopted by many workers for this ubiquitous proteolytic non-pathogenic form, and while it may not be entirely beyond criticism, it is a much better appellation than the one adopted by myself.

The literature of the anaerobes is full of obscurities owing to the very great difficulty of obtaining really pure cultures; this complicates the naming and placing of the forms obtained and causes considerable confusion.

TABLE I.

Number of days since date of wounding.	No anaerobes grown.	Anaerobes other than round endsporers.	Round endsporers present but cultures not toxic.	Round endsporers present including *B. tetani*, cultures toxic.	Total.	Percentage of cases with round endsporers.
Within 7 days of wounding	3	9	8	2	22	45%
Between 7th and 15th day	11	15	7	0	33	21·2%
Between 15th and 30th day	14	15	16	0	45	35·5%
Between 30th and 60th day	33	41	10	7	91	18·7%
Between 60th and 90th day	18	19	10	0	47	21·2%

TABLE II.

PERCENTAGES OF THE DIFFERENT CLASSES OF CASES IN THE FIRST AND SECOND MONTHS OF WOUNDING.

	No anaerobes grown.	Anaerobes other than round endspores.	Round endspores present but cultures not toxic.	Round endspores present including *B. tetani* cultures toxic.	Actual number of cases examined.
During first month	28%	39%	31%	2%	100
During second month	36%	45%	18%	7·6%	91

II.—EXUDATES FROM CASES OF DECLARED TETANUS.

To pass now to the consideration of the exudates from cases of declared tetanus. I am indebted to the military surgeons and officers in charge of a large number of different hospitals for the samples sent, and I wish to take this opportunity of thanking them for their assistance in this matter.

A total of 45 cases was dealt with culturally; they were all from patients who, in the opinion of the medical officers in charge, were or had recently been suffering from tetanus intoxication. As the material came from a large number of different sources and often from a distance, there was more variation in the nature of the samples than in those of the previous series. In addition, the data as to the period of the disease at which the sample was taken, the treatment in use, etc., were sometimes rather scanty; in several cases the patient was convalescent or indeed had recovered entirely before the material was sent to me. The relevant information was got together in as many cases as possible, but there are nevertheless some gaps which could not be avoided.

The whole series of 45 was dealt with culturally in the manner described above. Eight of these failed to produce the growth of any anaerobes; 37 cases gave cultures containing anaerobes of one kind or another. It is interesting to note that 29, or 78·3 per cent. of all these cases that gave growth, shewed the round endsporing type. Now I am not prepared, even in toxic cultures derived from cases of declared tetanus, to consider that a round endsporer must necessarily be *B. tetani*. What is important is to note that the condition of wound favourable to the production of a case of tetanus will give cultures with round endsporers in at least 78 per cent. of the cases. The criticisms expressed above as to the slight irregularities in the collection of the samples and the occurrence of convalescents in various stages of recovery must be borne in mind in this connection. They tend to lower the percentage of cases with round endsporers. The connection of the appearance of organisms with round endspores in the culture from the exudate and the wound condition favourable to the persistence of *B. tetani* was already shewn in the earlier part of the paper; the complementary nature of the two sets of data is obvious.

In 22 cases, out of a total of 45, part of the exudate was inoculated directly into a mouse without cultivation; in only two cases was there a positive tetanus reaction produced, namely in Nos. 1 and 121. No. 1 shewed toxin also in the subsequent cultures; No. 121 failed to produce growth upon anaerobic cultivation. In six of the cases in which direct inoculation failed to produce a tetanus reaction, an actively toxic culture was obtained upon cultivation of the same sample. One is therefore led to conclude that a direct inoculation of wound exudate into a single mouse is not of much use as a means of determining if *B. tetani* is present or not.

A group of 23 cases, out of a series of declared tetanus, were dealt with in exactly the same way as the indifferent cases described in the first part of the paper, and were tested for toxicity by inoculation of the culture into animals. Twelve of these produced toxic cultures; in four of these the exudate was obtained from patients who ultimately succumbed to the disease. Out of the 11 cases where toxin was not exhibited in the cultures, it was found upon examining the data available that seven were definitely convalescent after treatment with antitoxin when the sample was taken: one was recorded as being a case where the symptoms were slight, and in the three remaining I could not obtain the detailed history of the case.

Certain of the cases tested were of particular interest.

Case 33.—Develops tetanus in the seventh week from date of wounding; receives energetic treatment with antitoxin, receiving a total of 57,000 U.S.A. units in three days. The sample taken the day after treatment was discontinued, developed no toxin upon culture.

Case 211.—Develops tetanus in the second week; he receives a total of 45,000 units of antitoxin in three days. The sample taken two days after the treatment was discontinued, developed no toxin upon culture.

Case 20.—Develops tetanus in the fourth week; he receives 55,000 units in two days. The sample taken seven days after the treatment was discontinued, developed no toxin upon culture.

In these three cases at the end of treatment with antitoxin, it was not possible to demonstrate living *B. tetani* in the wound exudate. The treatment involved large doses of antitoxin.

Case 3.—Develops tetanus in the fourth week; he receives 2,500 units in four days. The sample taken four days after the treatment with antitoxin was discontinued, developed no toxin upon culture.

Case 39.—Develops tetanus in the second week: he receives 12,000 units in four days. The sample taken two days after the treatment was discontinued, developed no toxin.

In these cases the doses of antitoxin were less massive, and here also the wound exudate at the end of the treatment failed to shew living *B. tetani.* At the termination of the treatment in the five cases cited, the patient is left in a condition which appears to place him beyond the danger of a recurrence of tetanus.

Case 220.—Develops tetanus in the eleventh week; receives 5,000 units intrathecally in two days. The sample taken on the day the treatment is discontinued, produced a toxic culture.

Case 114.—Develops tetanus in the eighth week; receives 16,000 units intrathecally in two days. The sample taken on the day the treatment is discontinued, produced a toxic culture.

Case 265.—Develops tetanus on the tenth week; receives 4,500 units subcutaneously in five days. The sample taken five days after the treatment with antitoxin was discontinued, produced a toxic culture.

Case 252.—Develops tetanus in the fourth week; the symptoms are slight. On the third day after onset of symptoms, patient gets 500 units intrathecally and 500 units intravenously: the injections are discontinued as the patient develops pneumonia, from which he recovers. The sample wound exudate taken eighteen days after the treatment with antitoxin is discontinued, produces a toxic culture.

All the four last-cited cases recovered from the attack of tetanus.

Cases 114 and 220 do not call for any particular remark; living *B. tetani* could be demonstrated in the wound at the end of treatment, but the samples were taken immediately upon the termination of the injection of antitoxin and the elimination of the organism might not yet have been effected. Case 265 is interesting, as five days after the injection of antitoxin is discontinued, he still harbours living *B. tetani.* The patient recovers, but he was not definitely placed beyond the possibility of a return of the symptoms by the treatment adopted. In Case 252 this is still more evident; here the patient's wound still contained living *B. tetani* eighteen days after the antitoxin was discontinued. The symptoms had been suppressed, but the treatment had not eliminated the organism, and the patient was still in danger of a possible return of the disease. The importance of the condition in which cases like 265 and 252 are left at the end of the antitoxin treatment, is shewn by the history of such a case as 199. The patient has an attack of tetanus beginning on the eighth day after wounding, this is dealt with in France by the injection of tetanus antitoxin, and the patient recovers. In the ninth week after wounding, tetanus symptoms arise for the second time. He receives nine subcutaneous injections spaced over twenty-five days, the total number of units administered being 18,250. A sample of the wound exudate taken three days after the fourth injection, at a time when the patient had received 11,500 units, did not produce toxin upon cultivation.

Considering both the indifferent series and the series of declared tetanus, there seems to be a perfect graduation between the cases such as 27, 62 and 68, where living *B. tetani* can be obtained from the wound in the seventh and eighth week after wounding, but in which there are no symptoms of intoxication, through cases with slight symptoms like 252, to the rapidly fatal case like, for instance, 299, where the patient died within 24 hours of the onset of symptoms.

I wish, in conclusion, to express my thanks to Dr. HELEN CHAMBERS, Dr. ST. JOHN BROOKS, Capt. BALL, R.A.M.C., and Dr. ATKIN, for very kind co-operation in the collection of the material.

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APPENDIX.

Cooked Meat Medium.—Eight ounces of bullock's heart minced finely and then ground in a mortar ; add eight ounces of tap-water and heat slowly so as to cook the meat thoroughly : add normal sodium hydrate until the mixture is markedly alkaline to litmus. Divide into tubes and autoclave at 120° C. for half-an-hour. Particular care should be taken to see that the reaction is definitely alkaline at the time of inoculation, a useful rough test is afforded by the colour of the medium, it should be brown. Care must be taken in the sterilizing of the medium ; it should be borne in mind that the meat may contain many very resistant spores, and it is quite essential that the medium should be exposed to the actual temperature of 120° C. at least for half-an-hour.

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3. **The Distribution among Foodstuffs (especially those suitable for the rationing of Armies) of the substances required for the prevention of** *(a)* **Beriberi and** *(b)* **Scurvy.** By HARRIETTE CHICK and MARGARET HUME. *Transactions of the Society of Tropical Medicine and Hygiene*, Vol. X., 1917.

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5. **The Alleged Antineuritic properties of** *a*-**Hydroxypyridine and Adenine.** By A. HARDEN and S. S. ZILVA. *Biochemical Journal*, Vol. XI., 1917.

6. **On the Occurrence of Hydatid Cysts in Monkeys.** By W. NICOLL. *Parasitology*, Vol. X., 1918.

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A SIMPLE MEANS OF ASCERTAINING IF A STERILIZING HUT IS HOT ENOUGH TO DESTROY LICE AND NITS IN CLOTHING OR BLANKETS.

By A. BACOT,

ENTOMOLOGIST TO THE LISTER INSTITUTE OF PREVENTIVE MEDICINE.

The following method was planned in the first instance with a view to its possible service to sanitary officers who had to use extemporized sterilizing chambers, but a subsequent knowledge of the working of permanent hot-air sterilizing huts in home camps suggests that it might be of assistance to officers in charge of these also. Unless an electric ventilating fan, or other efficient means of circulating the air and so causing a uniform temperature throughout the chamber, exists, a stratified condition obtains, in which the heat is too low to kill at the lower levels and needlessly high at the top. For instance, door thermometers (that is, thermometers visible through a glass panel let into the door) record only the temperature in the vicinity at the level at which they are placed, and Captain J. T. Grant's trials with maximum thermometers have shown that, while the door thermometer records 60° C., the temperature just over the floor may be only 37° C., while at 7 ft. it is 103° C. The use of maximum thermometers placed at different situations within the chamber affords a useful check upon the record of the door thermometer, but may be deceptive, as only the extreme temperature is registered, there being no indication of the length of the period to which the clothes have been exposed to it.

By the use of porcelain pots or dishes of a definite surface area, containing a given quantity of stearin or paraffin wax

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of a suitable melting point, a sufficiently stable relationship between the heat and period required to kill both the insects and their nits can be established. In arriving at this relation a number of experimental trials must be made, but once the quantity and surface area required for a stearin or wax of a known melting point is settled only the simplest precautions are required to ensure efficiency. It is necessary that the wax or stearin should be replaced when it gets dirty, as otherwise its melting point may be reduced, and that the pots, which may either stand or hang, are not tilted at such an angle that the surface area is altered. Even if this last condition should occur, however, it will lead to an increased temperature or period of exposure being needed to melt the stearin, so that the error would be on the safe side.

I have worked out the following quantities in relation to the temperature and period needed for the destruction of the nits of *Pediculus humanus* when enclosed in a pocket made of khaki cloth as used for army breeches in France and the home service. The samples of stearin used were supplied by Messrs. Price, Ltd., and melted at 60° C., according to the trade tests. Nits, when protected by a single thickness of khaki cloth as above described, are killed by a fifteen minutes' exposure to a temperature of 52° C. As the method pursued in practice is to place clothing or blankets in a cool, or with the second and later batches in a warm, chamber, and count the exposure period from the moment when the door thermometer reaches the stated temperature, the same procedure was followed in the trial tests. The periods quoted, therefore, always presuppose a gradual rise up to the temperature, except in the case of the precautionary tests dealing with a quick rise to a high temperature mentioned below. In order to allow an adequate margin for contingencies, the temperature and period required for the destruction of nits is assumed to be 60° C. for thirty minutes, thus allowing as a margin at least fifteen minutes in time and 8° C. The porcelain pots used were supplied by Messrs. Price, Ltd.; they are those commonly used in the trade for samples of tallow, stearin, etc., and should therefore be easily obtainable. They are 2½ in. deep by 2½ in. in diameter; if only smaller or larger ones are obtainable the quantities of stearin used must be reduced or increased accordingly. The stearin must, of course, be melted in the pot and allowed to cool before use; 7 grams of stearin (trade melting point 60° C.) require thirty minutes at 60° C. under the conditions named, a small portion being still unmelted after twenty-five minutes; 10 grams require between forty and fifty minutes to completely melt it, only a narrow ring being melted within thirty minutes.

If two pots, one containing 7 grams and the other containing 10 grams, are placed or hung slightly below the level of the lowest garments in the sterilizing room, one can be sure, if all the stearin in the 7-gram pot is melted before the removal of the garments, that the exposure has been sufficient, both as regards period and heat; while, if all the stearin is melted in the pot containing 10 grams, it will show that greater heat or a longer exposure than was necessary has been used.

The question of the possibilities of an altered relation between the melting of the stearin and the killing of the nits with a short-period exposure to a higher temperature was tried. Nits and stearin were exposed together, and it was found that the stearin was more resistant to these conditions than the nits. For instance, when the temperature was rapidly raised from 21° C. to 80° C. within twenty minutes, the nits were killed while the 7 grams of stearin was not quite all melted. A rise to the same temperature in eighteen minutes showed the same result. A rise to 82° C. in fifteen minutes was just sufficient to melt all the stearin, the nits being killed. Again, a rise in twelve minutes to 81° C. killed the nits, but left a central disc of stearin unmelted.

Author's Presentation Copy

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A NOTE ON THE PERIOD DURING WHICH THE EGGS OF *STEGOMYIA FASCIATA* (*AËDES CALOPUS*) FROM SIERRA LEONE STOCK RETAIN THEIR VITALITY IN A HUMID TEMPERATURE[1].

By A. BACOT, F.E.S.

(*Entomologist to the Lister Institute.*)

It is well known that the eggs of the above-named mosquito remain viable when out of water for many months. Various workers, among whom may be named Theobald, Newstead, Francis and Mitchell, record varying periods up to nine months' duration.

Howard, Dyar and Knab (1912) state that with some species of *Aëdes* the eggs may remain dormant over a year. It does not seem to be so generally recognised, however, that the eggs *when immersed in water* may remain dormant. Marchoux, Salimbeni and Simond (1903), and Dupree, according to Mitchell (1907), were acquainted with the fact. The French investigators record a period of 70 days and Mitchell one of over a year. I think, Goeldi, as quoted by Howard, Dyar and Knab (1912), must have encountered the phenomenon but he considered the dormant eggs to be dead, at least it is in this light that I view his statement that submerged eggs perish. Bacot (1916) showed that immersed eggs may remain dormant for five months. It is apparently essential to the retention of vitality under conditions of drought and dormancy, when submerged, that the eggs shall have been incubated, development within the egg having proceeded up to the stage of the larva being ready for immediate hatching.

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[1] Since this Note went to press a batch of some 2000 or 3000 eggs of this mosquito, laid during April 1916, was tested after 15 months' storage. The eggs had been laid on filter paper and were placed in a wax card jar kept in the cool room (ca. 9° C.) of the Lister Institute. All the eggs were placed in a pan of tap water; a few hatched within 24 hours, and others responded later to the stimulus of the addition of a little *B. coli* culture to the water. Twenty adults were reared.

So much by way of preface seems needful in explanation of the following detached notes.

On my return from Freetown in August, 1915, I brought home a batch of ova of *Stegomyia fasciata*, and from these eggs reared adult mosquitoes. During November, 1915, a large supply of eggs was obtained with the object of ascertaining the extreme period of viability of eggs stored out of water but in a humid atmosphere.

The eggs, laid on filter paper, were placed in a waxed card jar with a waxed paper cover and kept in an unused ice chest in a cool cellar. This cellar has a bricked but uncemented floor and shows a very even range of conditions; the temperature seldom showing more than one or two degrees' variation between night and day, while the percentage of humidity is always high. Although circumstances did not permit of constant readings during 1915 and 1916, a fairly complete daily record exists for the period between November, 1910, and February, 1912. Monthly averages based on this, together with a chart showing curves of maximum and minimum temperatures and the humidity are published in the Plague Supplement III of the *Journal of Hygiene*, 14th January, 1914, pp. 456 and 461. From this it will be seen that the fluctuations of temperature within a period of 16 months lie between 6·6° C. (44° F.) to 17·8° C. (64° F.) while the record of humidity is even more stable, the monthly averages only varying between 94 and 95 %.

Some thousands of eggs were stored and the numbers used in the experiments were correspondingly large. Time did not permit of detailed counting, but the round figures given may be taken as approximately correct, being the outcome of estimates, backed by considerable experience of actual counts.

The immersion tests of the eggs were commenced 7 months after laying.

Tested after	Result
7 months.	Eggs hatched readily during July, 1916, probably not more than 10–20 % of the larvae failing to emerge.
9 ,,	Eggs hatched readily on 31 August, 1916.
10 ,,	On 2 October, 1916, a very noticeable rise in the percentage of failures to hatch was observed. Some of the larvae, however, quitted their eggs within two hours of immersion.
11 ,,	On 4 November, 1916, only a few of the eggs immersed yielded larvae; probably not more than 5 %, yet some of these quitted the shells within an hour of immersion. Four adults were reared within 10 days and 8 more within 12 days.
12 ,,	On 4 December, 1916, 5 larvae emerged from about 600 eggs, but none earlier than 5 or 6 hours after immersion. All the larvae were full

grown within 7 days, and two male specimens were reared within 10 days, the other three took longer, but the most backward larvae had pupated by the 9th day.

Over 13 months. On 12 January, 1917, a single larva emerged from a batch of about 1000 eggs. It hatched within 24 hours of immersion and pupated within 7 days; a male specimen was reared within 10 days.

On 15 January, 1917, some 14 to 15 months after laying, the remainder of the eggs (upwards of 1000) were immersed, but all failed to hatch.

It is of course possible that the temperature conditions were unfavourably low during the storage of the eggs for a race of the insect occurring in West Africa. Although none of the eggs stored out of water by Bacot (1916) in Freetown retained their vitality for more than 8–9 months, it might be objected that this was owing to the humidity factor being unfavourable and that in a saturated atmosphere a higher temperature than that of the cellar used in this experiment would allow of an extension of the period of viability. As against this, the facts concerning the period of viability when immersed must be considered. The longest period of dormancy noted in regard to submerged eggs under natural conditions (in West Africa) was 5 months, and although in an incubator at 21° C. (75° F.), in London, I have had several equally long records of dormancy, and one of 7 months, the conditions were admittedly unnatural in that the eggs had been externally sterilized and then placed in sterile fluids.

Mitchell's record (1917, p. 26) relating to the survival of the eggs when immersed in water for over a year, is unfortunately unaccompanied by any details that suggest close observation. In another passage (p. 148) this author places the limit at 9 months, while the looseness of phraseology as indicated by the statement "as the single eggs will resist drying almost indefinitely" leaves one in doubt as to whether the periods mentioned are the result of exact observation or conclusions based on general experience.

I venture to call the attention of American investigators to the desirability of further evidence on this point. Though I am prepared to find that the period may be longer than my evidence shows, it seems to me possible that the African and American races of *S. fasciata*—to suggest no smaller division—may differ considerably in constitution. Personally I am inclined to consider that the cool conditions did not affect the viability of the eggs experimented with and that so far as eggs of West African origin are concerned, the extreme period of viability of eggs stored out of water may be taken to be about a year.

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THE DISTRIBUTION AMONG FOODSTUFFS (ESPECIALLY THOSE SUITABLE FOR THE RATIONING OF ARMIES) OF THE SUBSTANCES REQUIRED FOR THE PREVENTION OF *(A)* BERIBERI AND *(B)* SCURVY.

BY HARRIETTE CHICK AND MARGARET HUME,

From the Lister Institute of Preventive Medicine.

(Paper read at a Meeting of the Society of Tropical Medicine and Hygiene, Friday, February 16th, 1917.)

NOTE.—This paper has been unavoidably held back. The authors have taken the opportunity to make some additions, *e.g.*, references to the Mesopotamia Commission Report and an Appendix.

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IV. RELATION BETWEEN BERIBERI AND SCURVY AS REGARDS TIME OF ONSET.

I. INTRODUCTION.

Sufficient evidence is now available to permit beriberi and scurvy to be classed among the "deficiency" diseases due to a defective diet without further comment. The view that scurvy is caused by the absence of fresh food, especially vegetables, was established in popular opinion more than 150 years ago,* and is maintained to-day, in spite of various attempts, some of which are quite recent (*e.g.*, JACKSON and HARLEY, 1900, and COPLANS, 1904), to suggest and defend a different etiology. The study of beriberi is confined to more recent times, and, during the last twenty years, has received the attention of many capable investigators. The researches of **EIJKMAN**, **GRIJNS**, **BRADDON**, and FRASER and STANTON, among others, have proved beriberi among the rice-eating populations of the East Indies, where it is prevalent, to be due to a definite deficiency in the diet of the persons afflicted. The defect was traced to a loss of specially valuable constituents of the rice grain which occurred during milling and polishing after the modern method, and these workers showed that the disease could be prevented if unmilled rice were substituted for milled rice in the diet, or if the bran removed during milling were added to the polished rice. The later workers on this subject include SCHAUMANN (1910) and FUNK (1913), in whose papers a complete bibliography of the subject may be found. To FUNK (1912) we owe the expression "vitamine," which he invented to express the unknown essential principle whose absence from a diet occasions beriberi. The term has, however, become established in general use to signify any accessory substance necessary for satisfactory metabolism, deficiency of which in a diet will lead to the occurrence of a "deficiency" disease.

The present position may be summed up as follows: For perfect nutrition the human being requires—in addition to an adequate ration of fat, protein, carbohydrate, salts and water—a sufficient supply of accessory food factor or vitamines. These substances have not so far been successfully isolated; little is known of their chemical or physical properties, and, at the present time, their presence can only be detected by biological methods. There are, at least, two[1] distinct classes of these vitamines:

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*JAMES LIND. "A Treatise on the Scurvy." 2nd ed. London. 1757.

[1] Rickets and pellagra, and some less known diseases of cattle, are also generally classed among the diseases due to defective nutrition: investigation in these cases is, however, not nearly so far advanced as in scurvy and beriberi.

(1) the vitamine whose presence in a diet is an essential for the proper nutrition of the nervous system, and whose absence or deficiency will give rise to beriberi—for the sake of brevity this may be called the anti-neuritic or anti-beriberi vitamine, and (2) the anti-scorbutic vitamine, whose absence or deficiency in a diet will occasion scurvy with its characteristic pathological changes.*

Scurvy and beriberi are now known to be respectively caused by a deficiency in these two different classes of vitamine, which possess different properties, play a different part in metabolism, and are differently distributed among food-stuffs in nature.

Deficiency diseases are practically non-existent among modern civilised Europeans, for, when living upon the ordinary mixed diet of civilisation, it is difficult to avoid getting an adequate amount of each separate vitamine. It is where the diet is more simple, as is the case with many Eastern races, that the risk of deficiency disease becomes proportionately greater. The food then needs careful scrutiny to ensure that all necessary vitamines should be included in the comparatively few substances of which the diet is composed.

In the case of Europeans, however, a variety of special causes may lead to what is virtually a restricted diet, *qua* vitamines, although it may not be apparent. Armies on active service, exploration parties in arctic regions, may enjoy a varied supply of preserved and sterilised foods but in effect be deprived of vitamines, these being wholly or in part destroyed by the high temperatures and other processes to which the food has been subjected in the course of preparation. Under such conditions beriberi or scurvy may take some months to develop to an acute stage, but there is little doubt that the defective diet will give rise to a general condition of ill health and inefficiency some time before definite symptoms of these diseases can be diagnosed by the physician. It is clearly of the utmost importance to ensure that a satisfactory diet should be available for soldiers and sailors on active service, more especially when one considers the great strain, both mental and physical, to which they are subjected. The first step towards this achievement is to obtain trustworthy information as to the distribution of these essential vitamines in ordinary foodstuffs. Some of this knowledge is already available, and it was with the hope of supplementing the existing data upon the subject that the present work was undertaken.

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*The spongy and septic condition of the gums characteristic of scurvy is probably partly, if not wholly, due to secondary bacterial infection. The defences against invasion are well known to be weaker in the mouth than in many other parts of the body, and in scurvy are further reduced owing to the abnormal condition of the blood system. This may account for the view frequently advanced, with some experimental support, that scurvy is an infectious disease of bacterial origin.

II. EXPERIMENTAL.

1. DISTRIBUTION OF ANTI-BERIBERI VITAMINE.

The distribution of the anti-beriberi vitamine among various foodstuffs was made the subject of a special investigation by **Cooper** (1913 and 1914), working in this Institute. We have used his methods, and our endeavour has been to extend his work with special reference to substances which are dry and portable, and hence specially suited to the rationing of troops on active service.

The deficiency polyneuritis of birds (which is readily induced experimentally by a diet of polished rice, see Fig. 1) has been accepted as analogous to human beriberi from the similarity of its etiology, symptoms and method of cure. **Cooper** chiefly used the pigeon as experimental animal. This bird is specially adapted for the study of the anti-neuritic vitamines, seeing that it is extremely susceptible to polyneuritis, but does not apparently take scurvy, even when deprived of anti-scorbutic material. Our work has also been done with the pigeon, and, following the methods of **Cooper**, we have studied the anti-neuritic properties of a series of foodstuffs by means of the following two types of experiments :—

A. Preventive Experiments.—In which the aim was to determine the minimum amount of the foodstuff that must be added to a vitamine-free diet to prevent onset of polyneuritis. Polished rice (40 grams daily) formed the vitamine-free diet, and prevention was reckoned to be successfully accomplished if the bird shewed no symptoms after 50-60 days. Unprotected birds usually develop acute polyneuritis after 15-25 days.

B. Curative Experiments.—In which the birds were fed upon polished rice until acute symptoms of polyneuritis were observed. Determination was then made of the minimum amount of the foodstuff in question which would effect a cure when administered by the mouth. Birds suffering from acute polyneuritis will usually die within twenty-four hours if not treated. In order to get the requisite amount of curative material (anti-beriberi vitamine) absorbed in time, it was frequently necessary to concentrate the vitamine. To do this a weighed quantity of the air-dry material was extracted with alcohol by shaking up with alcohol in the cold for some hours. The alcohol was then evaporated at low temperature under reduced pressure, and the residue was taken up in a small measured quantity of water. Definite amounts of this watery solution formed the curative doses, but in order to obtain a fairer comparison the amount of the dose was reckoned in terms of the original foodstuff.

TABLE I.

PREVENTIVE EXPERIMENTS.

Minimum daily ration which must be added to a diet of polished rice to prevent onset of Polyneuritis (Beriberi) in a pigeon of 300 to 400 grams weight.

SUBSTANCE.	DAILY RATION.		OBSERVER.	NOTES ON RESULTS.
	Natural foodstuff. (Grms.)	Dry Weight. (Grms.)		
Wheat Germ, sample R.I., free from bran ...	1·5	1·3	H.C. & E.M.H.	Complete protection.
Wheat bran, sample R.I., free from germ ..	More than 1·5	More than 1·4	,, ,,	No protection with 1·5 gms. daily. Polyneuritis occurred in same time as in control birds.
,, ,,	More than 2·5	More than 2·2	,, ,,	Small degree of protection. Mean time of onset delayed about two weeks.
Yeast extract, commercial, sample A	0·5	0·35	,, ,,	Protection not secured in all cases.
	1·0	0·7	,, ,,	Protection.
Pressed yeast .	2·5	0·5	COOPER, *Journ. Hyg.* 1913 and 14	
Lentils	—	3·0	,, ,,	
Barley, unhusked	3·7	3·2	,, ,,	
Barley, husked	5·0	4·5	,, ,,	
Egg-yolk	3	1·5	,, ,,	
Beef-muscle...	20	5.0	,, ,,	
Ox heart-muscle	5	1·7	,, ,,	
Ox brain	6	1·2	,, ,,	
Ox liver	3	0·9	,, ,,	
Sheep brain...	12	2·5	,, ,,	
Fish-muscle...	More than 10	More than 2	,, ,,	
Cheese	More than 8	More than 5·6	,, ,,	Protection not secured with these amounts.
Cow's milk	,, ,, 35	,, ,, 3·5	,, ,,	

TABLE II.

CURATIVE EXPERIMENTS.

Minimum amounts of foodstuffs required to cure Polyneuritis (Beriberi) in a pigeon 300 to 400 grams weight. The doses are reckoned in terms of the original foodstuffs.

SUBSTANCE.	Preparation of curative material.	Amount of dose given.		RESULT.	OBSERVER.
		In terms of the natural foodstuff.	In terms of dry weight 100–110° C.		
		grams.	grams.		
Wheat germ—					
Commercial sample, cooked	Extracted with alcohol	8	6·9	Improvement	H.C. & E.M.H.
		12	10·3	Sometimes cures	,, ,,
		16	13·8	Cure	,, ,,
Sample A "picked," uncooked	Extracted with alcohol	10	8·7	Incomplete cure	,, ,,
		15	13·0	Complete cure	,, ,,
Sample B "picked," uncooked	Unextracted	1·0	0·9	Sometimes cures	,, ,,
		2·5	2·2	Cure	,, ,,
*Maize germ	Unextracted	1·0 to 3·0	—	Cure	,, ,,
*Rice germ	Unextracted	0·5 to 1·0	—	Cure	,, ,,
Wheat bran—					
Stone ground, not free from grm.	Unextracted	5·0		Sometimes cures	,, ,,
Roller milled, free from germ	Unextracted	3·0	—	No cure	,, ,,
		5·0	—	Sometimes cures	,, ,,
Rice bran, containing germ	Unextracted	6·0	—	No cure	,, ,,
		10·0	—	Complete cure	,, ,,
Turbot fish-roe (hard)	Extracted with alcohol	35	10	Cure almost comp	,, ,,
		70	20	Cure complete	,, ,,
		140	40	Cure complete	,, ,,
Egg-yolk	Extracted with alcohol	60 = 4 yolks	30	Cure	Cooper, 1913.
Dried whole egg, com'l, samp. I.	Given without extraction	40 abt. 4 ,,	38	Cure	H.C. & E.M.H.
,, ,, ,, ,,	Do.	30 ,, 3 ,,	29	Cure	,, ,,
,, ,, ,, ,, II.	Do.	20 ,, 2 ,,	—	Cure	,, ,,
Malt Extract—					
1st sample	Given without extraction	5	4·2	Cure	Cooper, 1914, I.
2nd sample	Do.	7	5·1	Cure	,, ,,
3rd sample	Do.	10	9·2	No Cure	,, ,,
Meat extract, com'l, sample I.	Do.	3	2·8	No improvement, bird died	H.C. & E.M.H.
,, ,, ,, ,,	Do.	6·5	5·2	No improvement, bird died	,, ,,
Raw beef	Extracted with alcohol	140	30 approx.	Cure	Cooper, 1913.
"Maconochie" ration	Do.	440	106	Cure incomplete	H.C. & E.M.H.
"New ration" roast beef tinned, submitted for examination by the Dept. of Hygiene, R.A.M. College, on 19th June, 1916	Do.	350	112	Very slight improvem't, no cure	,, ,,

* Picked out from the un-milled grain by hand in the laboratory.

TABLE II.—*continued.*

CURATIVE EXPERIMENTS *continued.*

Minimum amounts of foodstuffs required to cure Polyneuritis (Beriberi) in a pigeon 300 to 400 grams weight. The doses are reckoned in terms of the original foodstuffs.

SUBSTANCE.	Preparation of curative material.	Amount of dose given.		RESULT.	OBSERVER.
		In terms of the natural foodstuff.	In terms of dry weight 100–110° C.		
Dried peas..	Extracted with alcohol ...	30	26	Incomplete cure	,, ,,
		40	35	Complete cure ..	,, ,,
,, ,, ...	Unextracted ...	10	9	Cure	,, ,,
Pea flour, kilned	Extracted with alcohol ...	80	–	Slight impr'vm't. No cure ..	,, ,,
Dried lentils	Do.	20	18	Cure	COOPER, 1913.
Dried vegetables, commercial Sample "Spring greens"	Do.	40	35	Cure	H.C. & E.M.H.
	Do.	120	105	Complete cure ..	.. ..
Potatoes, peelings	Do.	180	36	Died .. .	,, ..
,, ,, ...	Do.	630	126	Improvement, no cure . ..	,, ..
Potatoes, insides...	Do.	200	40	Incomplete cure	.. ..
,, ,, . .	Do.	350	70	Cure . ..	.. ,,
Pressed yeast	Autolysed .	2·0	0·4	Cured slowly .	,, ..
,, ,, ...	Do.	3·0 to 6 0	0·6 to 1·2	Cure . ..	COOPER, 1914, II.
Yeast extract, com'l, Sample A	Given without extraction ...	1·5 to 2·0	1·0 to 1·3	Complete cure ..	H.C. & E.M.H.
Dried fruits, currant	Extracted with alcohol ...	60	47	No improvement	.. ..
,, ,, ,,	Do.	19	16	No improvement	.. ..
.. .. dates .. .	Do.	26	21	Slight improv'nt. death delayed	.. ..
.. .. ..	Do.	26	21	Slight temporary improvement ..	.. ..
Proprietary article, dry powder, stated to contain vitamines in a concentrated form ...	Given without extraction ...	12		No improvt., died	.. ,
		31		Temp. improvement, no cure	.. ..

In case of cereals and some other materials rich in anti-neuritic vitamine, we gave the curative dose of the original foodstuff without preliminary extraction. By this means we avoided the loss of vitamine due to the process of extraction. This loss was very considerable; see, for example, the case of dry peas given in Table II., where the curative dose of unextracted material was 10 grams, but that estimated from experiments made with an extract was as high as 40 grams. The original material was administered, without extraction, whenever possible, in order to obtain a better absolute value for the vitamine-content of the foodstuff in the natural condition. The drawback of this method is that absorption is slower and one does not get the swift dramatic cures commonly obtained when the curative principle is given in a soluble form. But if care is taken to see that the animal's crop is empty before administering a cure, digestion will not be too slow to permit of sufficient vitamine being absorbed in time to save the bird.

There is no doubt that the most trustworthy results are obtained from the preventive tests. They are, however, very exacting and laborious, the feeding is artificial, and must be continued for about two months, with daily careful supervision. If the enquiry were limited to this type of experiment its scope would be seriously restricted. In considering the values obtained from curative experiments, allowance must be made for a large margin of error, seeing that it is impossible to control the onset of polyneuritis so that the type of symptom and the severity of the condition should always be the same at the time of administration of the cure.

The principal data obtained are set forth in Tables I. and II., in which also many of the results of COOPER *(loc. cit.)* are included for the sake of completeness. Some of the results given in these Tables were anticipated by the workers mentioned on page 150, but COOPER was the first to make a systematic attempt to obtain a comparative set of values for the different foodstuffs as regards ability to prevent beriberi.

From Tables I. and II. it is seen that the anti-beriberi vitamine is extremely widespread, and is found present to a greater or less degree in almost every natural foodstuff investigated. The principal sources of this vitamine, however, are at once seen to be in the seeds of plants or the eggs of animals, where it is probably stored[*] as a provision for the nutrition of the offspring during the early period of its existence.

From the practical point of view, the most important result is the fact that *one of the chief sources of the anti-beriberi vitamine should be in the seeds of plants, including, as this does, the cereals and edible pulses.* The

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[*] It is interesting in this connection to note that among a population upon the verge of beriberi owing to a defective diet, it is frequently the pregnant women who are the first to shew acute symptoms (see VEDDER. "Beriberi." London. 1913. p. 61).

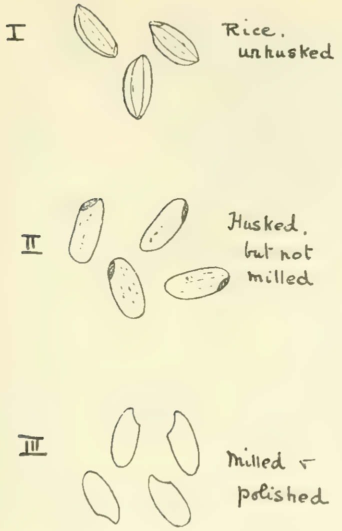


FIG. 1.

Shewing the various stages in Milling of the Rice Grain.

I. Rice grain in the natural condition, retaining the husk or enclosing glumes.

II. After removal of the husk, but retaining the pericarp or "silver-skin," and the embryo, which is shaded.

III. After milling and polishing; both "silver-skin" and embryo are removed and the grains are then "polished" by rubbing with talc between sheepskins.

FIG. 2.*

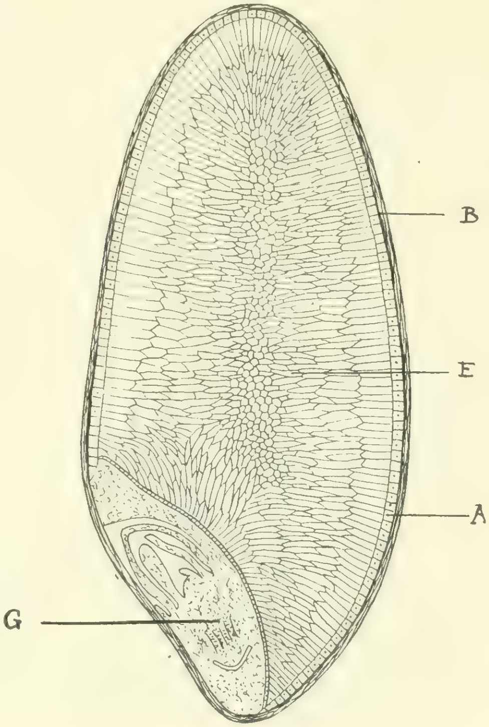
Diagram of a longitudinal section through a grain of wheat, shewing

B Pericarp, forming the branny envelope.

A Aleurone layer of cells forming the outermost layer of the endosperm removed with the pericarp during milling.

E Parenchymatous cells of the endosperm.

G Embryo or germ.



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*Reproduced, with the permission of the Controller of H.M. Stationery Office, from Figs. 1 and 2 in Dr. J. M. HAMILL'S "Report to the Local Government Board on the nutritive value of bread made from different varieties of wheat flour," 1911.

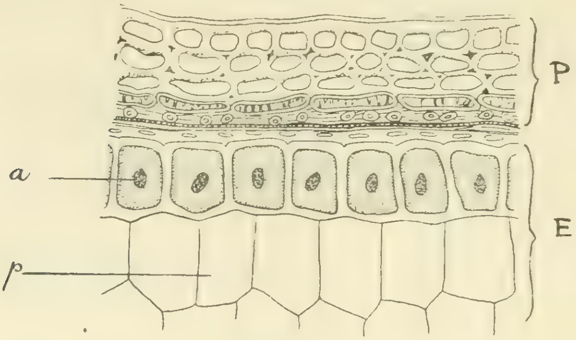

FIG. 3.*

Cross section through the branny envelope and outer portion of the endosperm of wheat grain, shewing—*P*, the pericarp; *E*, endosperm, consisting of *a*, layer of aleurone cells and *p*, parenchymatous cells.

*Reproduced, with the permission of the Controller of H.M. Stationery Office, from Figs. 1 and 2 in Dr. J. M. HAMILL's "Report to the Local Government Board on the nutritive value of bread made from different varieties of wheat flour," 1911.

great value of certain beans (*e.g.*, KATJANGIDJO, *Phaseolus radiatus*) in the prevention and cure of both avian polyneuritis and human beriberi, has been repeatedly shewn by GRIJNS (1901) and the other Dutch observers. In the case of cereals* an interesting differentiation exists in the different parts of the seed (see Figs. 1, 2 and 3). The largest deposit of the vitamine is present in the *embryo* or germ, while the bran (pericarp + *aleurone layer*) comes second in order of importance. The *endosperm* (especially when deprived of the *aleurone layer*, which is included in the bran in modern milling) is deficient in anti-neuritic vitamine, and will cause beriberi if employed as sole diet. One well-known example of a cereal endosperm is polished rice (see Fig. 1), where the germ is removed with bran during the milling and polishing. Another is ordinary white wheaten flour, in the preparation of which a similar deprivation takes place in the modern roller mills. In this case, during the "break" on the rollers, the oily germ is squeezed out flat, and subsequently can be separated completely from the flour and the bran. Both white flour and polished rice produce polyneuritis in pigeons in an exactly similar way, and, if the less complete evidence available in case of rye and maize be also taken into account, there is no doubt a general rule

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*A more detailed account of our experiments with cereals is published in the *Proc. Roy. Soc.*, 1917, read February 15th, 1917 (in the press).

that among cereals the anti-neuritic vitamine is concentrated in the germ.

The potency of the cereal embryo in the cure of polyneuritis is very great. In case of wheat germ, 2·5 grams usually (and rarely 1·0 gram) sufficed to cure a pigeon of 300-400 grams' weight in an acutely ill condition. In case of rice embryo, cures were obtained with amounts varying from 0·5 gram to 1·0 gram.

The prevailing opinion, hitherto, has been that the substance preventing beriberi was situated in the cuticle of the husked grain, in the layer of cells *(aleurone layer)* immediately below the pericarp (see Fig. 3). This view is based upon the well-known influence of decorticated rice in inducing human beriberi, and the prevention and cure of the disease by the addition of rice polishings and their extracts. The true explanation lies in the fact that in the milling and polishing of rice the germ is removed with the bran (see Fig. 1). On comparing the values of (1) commercial rice bran and (2) the pure germ, separated from the grain by hand in the laboratory, we found the latter ten times as potent as the former for the cure of pigeon polyneuritis.

An interesting analogy with the seeds and embryos of plants is offered by the eggs of birds and fishes, which also form valuable sources of anti-beriberi vitamine. The successful cures obtained with desiccated eggs shew the great resistance offered by this vitamine to drying.

Yeast is another substance rich in anti-neuritic properties, which it retains even after extraction or autolysis. Yeast was the only unicellular organism investigated, and it may be significant of the universality of this vitamine that it should be abundantly present in this instance.

Milk, cheese and potatoes appear to be among the least valuable foods from this point of view; other vegetables and meat contain a moderate amount of anti-beriberi vitamine, but are very inferior when compared with eggs or the seeds of plants.

2. DISTRIBUTION OF ANTI-SCORBUTIC VITAMINE.

While there is a good deal of empirical knowledge upon this subject, few accurate scientific data are available, and what exist are found chiefly in the work of AXEL HOLST and his colleagues Drs. FÜRST and FRÖLICH, in the University of Christiania (1907, 1912, 1913). By depriving guinea-pigs of fresh green food, and offering only a diet of grain and water, these workers were able to induce a disease analogous to human scurvy, from which the animal died within a month from the commencement of the restricted diet. The post-mortem appearances shewed all the lesions of typical scurvy, and included changes in bone, cartilage and bone marrow, loosening of the teeth, and, in addition, hæmorrhages, which might occur in any situation. These workers also studied the influence of various additions to this scurvy diet, and investigated the distribution of the anti-scorbutic principle among various vegetable foodstuffs and its resistance to drying and to exposure to high temperatures. The commonly accepted view that fresh vegetables and fruits are the chief source of anti-scorbutic vitamine was abundantly confirmed by their experimental work.

No symptoms suggesting beriberi were detected, nor were any nerve lesions discovered in these experimental animals, whose scurvy diet of various grains and water included unmilled cereals and contained abundant anti-neuritic vitamine. In any circumstances, the guinea-pig appears to be so highly susceptible to scurvy that attempts to produce beriberi have not been successful. The distribution of the anti-beriberi vitamine is very widespread, and on any diet adapted to this animal it seems impossible to protect satisfactorily from scurvy without at the same time supplying a sufficiency of the anti-neuritic substance. For this reason the guinea-pig is as admirably adapted for the study of scurvy as is the pigeon for that of beriberi.

During the last few months we have extended the work of HOLST and his colleagues in the direction of foodstuffs which are portable and convenient for transport, and hence suited for the rationing of armies. The methods we have followed have, in principle, been those of the Norwegian workers. The principal modification has been the addition of milk, subjected to prolonged heating at a high temperature (120° C. for one hour), to the scurvy diet of grain and water. By this addition we have not been able to detect any appreciable change in the special symptoms of the disease or time of onset; but the general condition of the animal is much improved, apart from the scurvy, and the influence of the inclusion of the anti-scorbutic substances can be studied with less complication from such factors as mere inanition and starvation (see Table III., and compare curves A and B in Fig. 4).

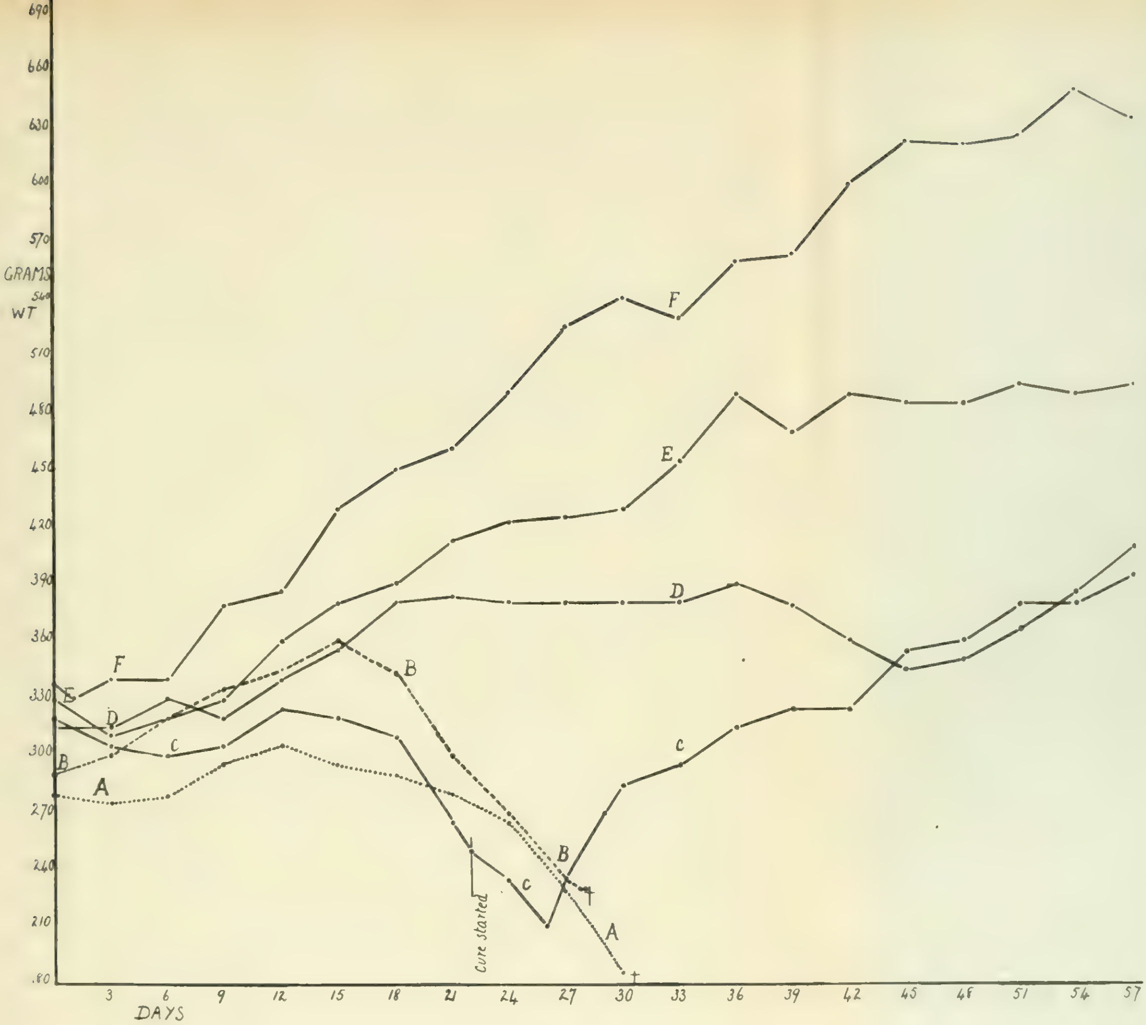

FIG. 4.

Weight Charts of five typical experiments shewing the anti-scorbutic value of orange juice and fresh cabbage leaves

CURVE A. Typical scurvy on a diet of oats, bran and water.

CURVE B. Typical scurvy on a diet of oats, bran and sterilised milk (heated to 120° C. for 1 hour in the autoclave).

CURVE C. Typical scurvy on a diet of oats, bran and water, cured by addition of orange juice and autoclaved milk to the diet on the 22nd day, when the symptoms were well marked.

CURVE D. Weight chart shewing influence of 5 c.c. fresh orange juice daily added to the "scurvy" diet; autoclaved milk added to the diet on the 56th day.

CURVE E. Normal weight chart on a diet of oats, bran and cabbage leaves (30 grams daily).

CURVE F Weight chart on diet of oats, bran, autoclaved milk and 3 c.c. fresh orange juice daily; specially favourable circumstances—warm weather, etc.

To face page 153

The experiments have almost invariably been of the preventive type. There is no particular advantage in the curative type of experiment, where scurvy is concerned. The onset of the disease is gradual, and by the time the symptoms—loss of weight, painful joints and loosening teeth—are well marked, the lesions are extensive and the animal is in a very weak condition. If at this stage a powerful anti-scorbutic, such as orange juice, is given, the cure is extremely slow, and it may be many weeks before the animal may be said to be in perfect health. In fact, it is doubtful whether the joints ever become perfectly normal, and even when the cured animals seemed to have reached a thriving condition, one could frequently detect a slight tenderness of the arm and leg joints by a careful examination.

The general course of these experiments may be seen from Fig. 4 and Table III., which give weight charts and brief protocols respectively of three typical cases.

Experiment I. (Curve A, Fig. 4) is a case of typical scurvy on a diet of oats, bran and water. The animal (280 grams' weight), which is in a young and growing condition at the beginning of the experiment, puts on no weight upon this diet. After seventeen days distinct tenderness of the joints is present, and from this time the weight begins to decline. After about another ten days the teeth are found to be loose, little food is taken, the loss of weight becomes very rapid, and death soon follows. The post-mortem appearances generally found in such cases are as follows:—Extensive hæmorrhages are found present in the muscles of leg and thigh, especially near the knee-joints; less severe ones are frequently found in the muscles of the fore limbs, round the axilla. Subcutaneous hæmorrhages are also frequently present, although not to be detected during life, owing to the thickness and pigmented nature of the skin. The bones are fragile and easily break off at the junction of shaft and epiphysis; this is specially true in the case of the tibia, which is often found to be already fractured. The jaws are very brittle and the teeth usually quite loose. There are marked swellings on the ribs at the bone-cartilage junction, which is often found to be fractured. On histological examination these junctions are found to be much disorganised and shew the characteristic changes in the bone marrow, so carefully studied and described by HOLST and FRÖLICH (1912).

Experiment II. (Curve B, Fig. 4) shews very clearly the improved condition when autoclaved milk is substituted for water in the diet, although the course of the scurvy is not modified. Weight is better maintained throughout, and, at the beginning of the experiment, some growth takes place.

Experiment III. (Curve C, Fig. 4) deals with a successful cure from scurvy by means of fresh orange juice. The first part of the experiment, on a diet of oats, bran and water, reproduces Experiment I. very closely. On the 22nd day, when the guinea-pig is losing weight rapidly and shews acutely sore joints, orange juice (10 c.c. daily) is added to the diet, and 50-60 grams of autoclaved milk. Little improvement is seen for four or five days, and the fall in weight continues. After this, however, a gradual improvement takes place, but it is 6-7 weeks after the beginning of the "cure" before the animal may be considered normal. The soreness of the joints persists long after the general health and weight have been restored.

The Curves D, E, and F, Fig. 4, shew the weight curves on diets which give adequate protection from scurvy, and are included for purposes of comparison. Curve E shews the increase of weight upon a normal diet of oats, bran and cabbage leaves (30 grams daily); and Curves D and F shew the result of substituting fresh orange juice for the cabbage leaves, with the addition also of autoclaved milk.

TABLE III.

EXPERIMENT I. (Guinea-pig 47). Diet: Oats, Bran and Water *ad lib.* (see Curve A, Fig. 4).

Date.	No. of days from beginning of Experiment.	Condition of the Animal.	Weight (Grams).	Amount of oats and bran eaten daily, average (Grams).
29-11-16	0		280	
16-12-16	17	Leg joints tender	290	(21 days) 34.
20-12-16	21		280	
25-12-16	26	Leg joints tender, incisor teeth loose	240	(9 days) 14.
29-12-16	30	Died	185	

EXPERIMENT II. (Guinea-pig 49). Diet: Oats, Bran and Autoclaved Milk (1 hour at 120° C.) *ad lib.* (see Curve B, Fig 4).

29-11-16	0		290	(17 days) 25.
15-12-16	16	One leg joint tender	350	
25-12-16	26	Teeth loose	240	(12 days) 5.
27-12-16	28	Died	230	

TABLE III.—*continued.*

EXPERIMENT III. (Guinea-pig 58). Diet: Oats, Bran and Water *ad lib.* for 22 days, with production of acute Scurvy; followed by the addition of Orange Juice, 10 c.c. daily, and Autoclaved Milk, about 50 grams daily, leading to a satisfactory cure (see Curve C, Fig. 4).

Date.	No of days from beginning of Experiment.	Condition of the Animal.	Weight (Grams).	Amount of oats and bran eaten daily average (Grams).
6–12–16	0		320	
16–12–16	10	One leg sore	315	
22–12–16	16	"Scurvy position" noticed, the sore hind-leg is raised in the air while the animal lies down on the other three legs.	320	(18 days) 33.
24–12–16	18	Great fall in weight begins	320	
28–12–16	22	Animal very weak *Cure started*, 10 c.c. fresh orange juice + 50-60 grms. autoclaved milk daily added to oats & bran diet	250	(5 days) 10.
29–12–16	23	Condition very bad	235	
1–1–17	26	Still very sore	235	
3–1–17	28	Lying in "Scurvy position"	270	(10 days) 15.
5–1–17	31	Soreness of joints improved	290	
7–1–17	33		295	
13–1–17	39	General condition good—joints still sore	325	
24–1–17	50	Ditto	365	
13–2–17	60	Soreness much improved	460	(41 days) 45.
21–2–17	68	No symptoms	482	
27-2-17	74	Excellent health, no symptoms	540	

It will be gathered that these experiments are laborious and tedious. The guinea-pigs in most cases require a certain amount of hand feeding to gain assurance that the requisite doses of the materials studied are really taken, and much care is also needed to render the foodstuffs palatable, for many of those studied are far removed from the natural food of this animal, and different treatment is required in each separate case. Further, a good deal of skilled attention is necessary if the general health and weight of the animal are to be maintained, apart from the question of scurvy. Under these circumstances the output of work possible from any one worker is limited, and we have been fortunate in obtaining the assistance of the following workers to whom, in conjunction with ourselves, the investigation of the following foodstuffs has been entrusted:—

Miss RUTH SKELTON (fresh and dried vegetables and lime juice).

Dr. E. Marion Delf and Miss Olive Lodge (fresh fruit juices; pulses, soaked and germinated).

Our endeavour is to obtain accurate quantitative figures expressing the relative anti-scorbutic value of a large range of different foodstuffs. The work is at present incomplete, but enough information is available from our own results and those of the Norwegian workers (confirmed throughout by our own) to construct the following Table IV., in which a rough estimate is given of the comparative value of the various substances in the prevention of *(a)* beriberi and *(b)* scurvy.

Table IV.

VALUE OF FOODSTUFFS AS PREVENTIVE AGAINST SCURVY AND BERIBERI.

Foodstuffs.	Water content per cent. (Approx.)	Value against Beriberi.	Value against Scurvy.
Cereals—			
Whole grain, wheat		+ +	0
Endosperm, polished rice	10 to 13	0	0
,, white flour (wheat) ...			
Bran, *e.g.*, rice		+ +	0
.. ., wheat			
Germ or embryo, *e.g.*, rice... ..		+ + +	0
.. .. .. wheat ...			
Pulses—			
Whole, in dry condition	12	+ +	0
Germinated Pulses (or Cereals)...	50	+ +	+ + +
Vegetables—			
Potatoes	80	0	+ +
Fresh, *e.g.*, cabbage	90	+	+ + +
,, ,, onions			
.. .. carrot			
Desiccated vegetables	10 to 15	+	+ to 0 according to age
Pickled, *e.g.*, cabbage	—	—	0
Fruit Juice—			
Fresh, *e.g.* orange	90	—	+ + +
.. .. lemon			
Eggs—			
Fresh	70	+ +	—
Desiccated	6	+ +	0
Meat—			
Fresh	70	+	+
Tinned		0	0
Milk—			
Cow's, fresh	87	0	+ (slight)
Yeast—			
Pressed, autolysed	77	+ + +	0
Extract, commercial sample A .	30	+ + +	0

— Signifies not investigated.

It is at once evident that the distribution of the anti-scurvy vitamine is much more restricted than that of the anti-beriberi vitamine. There are here no special places where deposits are found of highly concentrated anti-scorbutic material, but it is present in all living (actively metabolic) tissues of plants and (to a much less degree) in those of animals.

The anti-scorbutic principle has not been found present in dried vegetables or in any dried seeds, such as cereals or pulses, diets of which form the classic means of producing the disease of scurvy. If, however, *these seeds are moistened and allowed to germinate, the anti-scorbutic principle is created anew with the beginnings of active cell life.* As far as our present knowledge goes, the presence of the anti-scorbutic vitamine is always associated with living tissues in which active metabolism is taking place. When viable seeds are in the dry, resting condition, all the *active* processes concerned with life and metabolism are temporarily suspended, and a disappearance of the anti-scorbutic principle accompanies this cessation. If the seed is moistened, rapid

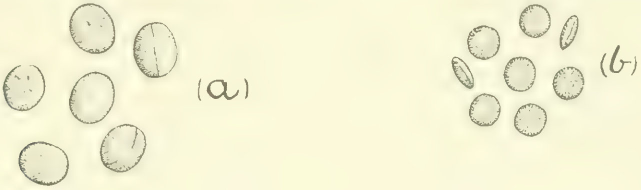

FIG. 5.

Pulses in the dry condition (natural size): *a*, Peas (*Pisum sativum*); *b*, Lentils (*Lens esculenta*).

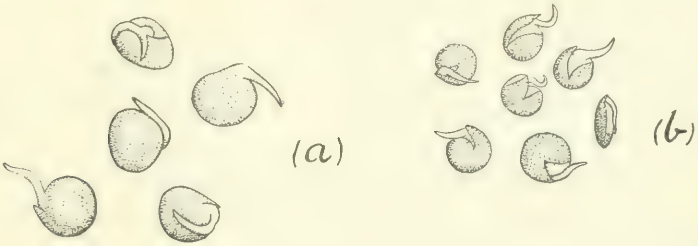

FIG. 6.

Same pulses, as in Fig. 5, after soaking in water for 24 hours and germination for 48 hours, at laboratory temperature (natural size): *a*, Peas (*Pisum sativum*); b, Lentils (*Lens esculenta*).

absorption of water takes place, the cells once more become turgid and the active processes associated with germination and growth will begin within a few hours. Accompanying this new activity the anti-scorbutic vitamine makes its appearance in the tissues and gradually increases in amount.

These facts were first discovered by Fürst (1912), and have been amply confirmed by ourselves and our colleagues in an extensive set of experiments with peas *(Pisum sativum)* and lentils *(Lens esculenta)*, see Fig. 5. Our method has been to soak these seeds in water for 24 hours, during which time they absorb their own weight of water. They are then loosely packed in a glass funnel and maintained in a moist atmosphere for 1-2 days longer, care been taken to ensure abundant access of air. After 48 hours at laboratory temperature (50-60 F.) the radicle is usually from 0·5 to 1·0 c.m. in length (see Fig. 6), and at this stage we have used the germinated seeds in our experimental diets. Our work is not yet sufficiently advanced to permit of a final statement of the anti-scorbutic value of these germinated pulses in terms of fresh vegetables, but we have obtained complete protection from scurvy with a daily ration of 5 grams, which indicates a vitamine-content comparable with that, for example, of fresh cabbage leaves.

Fruit Juice.—Fresh fruit juices are exceedingly rich in anti-scorbutic vitamine. Holst and his co-workers pointed out the value of fresh lemon and fresh raspberry juice in preventing experimental scurvy, and our researches have been made chiefly with orange and lemon juice. With these latter we have also been able to effect satisfactory cures from an acute scorbutic condition.

The results of our experiments with preserved fruit juice, *e.g.*, lime juice, have not been so encouraging, the anti-scorbutic power being very feeble in comparison with fresh juices. We are now investigating various methods of preservation in the hope that we may be able to suggest improvements. After reading the history of our Navy and Mercantile Marine, it is impossible to resist the conviction that, in former times, preserved fruit juice was of distinct value in preventing human scurvy, and it is not unlikely that modern methods of manufacture may have introduced some modification detrimental to the anti-scorbutic principle it originally contained.

Fresh Vegetables.—Among the fresh vegetables investigated, the cabbage was found best, partly, no doubt, because fresh green leaves happen to be the natural food of our experimental animal. Onions were also found to be powerfully anti-scorbutic, in spite of their dry appearance from the outside. Ordinary Spanish onions contain 95 per cent. of water, the cells are all in a turgid condition, and, botanically, the vegetable is to be regarded as a live bud. Potatoes and carrots must also be regarded as composed of live turgid tissue; they are also of great value, though found to be slightly inferior to cabbage leaves. In our experiments the potatoes were boiled before giving to the animals, and doubtless there was some loss of the anti-scorbutic principle during the process of cooking. It was, however, a necessary measure, as guinea-pigs are not able to take potatoes in the raw condition.

Milk.—Cow's milk possesses very low anti-scorbutic value. In our experience a ration of 50 grams daily was insufficient to protect guinea-pigs from scurvy. FRÖLICH (1912), however, found that they could be protected by an exclusive diet of milk, and we have found the same result if a minimum of 100 grams is taken daily. This is about twenty times the necessary daily ration (viz., 5 grams) of fresh cabbage or germinated peas, or fresh fruit juice. It seems probable that infants nourished on cow's milk do not obtain any excess of anti-scorbutic principle, especially if the milk is previously boiled. The modern custom of adding a small daily ration of fresh fruit juice, or other anti-scorbutic, appears to have sound and scientific foundation.

Meat.—It is practical experience, obtained especially from the history of Arctic exploration, that fresh meat will prevent human scurvy if taken regularly in fair quantity. The expressed juice of raw meat is also considered to be of value in the cure and prevention of infantile scurvy. For prevention of experimental guinea-pig scurvy, meat was found to be very disappointing, for little, if any, protection could be demonstrated. It must be remembered that meat is an unnatural food for guinea-pigs; they will not eat it even when cooked, and the experiments have to be made with the expressed juices. Allowance must also be made for the high susceptibility to scurvy of this animal, which is undoubtedly much greater than that of the human being; but, when all this is taken into account, one is forced to conclude that meat is far inferior as an anti-scorbutic to vegetables and fruit, and that the daily allowance must be large if reliance is to be placed upon it for the prevention of scurvy (see also below, p. 171).

Yeast.—Yeast Extract A and autolysed yeast, both of which were shewn to contain the anti-beriberi vitamine in high concentration, uninjured by the process of preparation, were found useless for the prevention of scurvy. Animals receiving as large a daily ration as could conveniently be taken, died of acute scurvy in the same period as the control animals on the "scurvy diet."

RESISTANCE OF THE ANTI-BERIBERI AND ANTI-SCORBUTIC VITAMINE RESPECTIVELY WHEN EXPOSED TO DRYING, HIGH TEMPERATURES, ETC.

(A) Drying.—From the common presence of the anti-beriberi vitamine among dry foodstuffs, it is evident that this substance is not sensitive to the process of drying. It is very significant that its chief sources should be found in dry seeds, such as cereals and pulses.

The case is otherwise with the anti-scorbutic vitamine. This principle seems to be *absent or deficient in all dry foodstuffs*, such, for example, as cereals and pulses, and to be rapidly destroyed when the animal and vegetable tissues, in which it is naturally contained, are subjected to drying. HOLST and FRÖLICH *(loc. cit.)* found dried vegetables of little use for the protection of guinea-pigs from scurvy, and we have confirmed their observations. A similar result has been noted in human experience. Dried vegetables were found useless in the epidemic of scurvy which ravaged the Austrian army in Hungary in the early part of the 18th century (BUDD, 1840), and, including dried potatoes, were tried with the same disappointing results during the American Civil War ("Medical History of the War of Rebellion," Vol. III., Washington).

The temperature at which the tissues are dried seems to be a matter of indifference. In HOLST and FRÖLICH'S experimental work (1912) low temperatures were employed, not exceeding 37° C. The destruction of the anti-scorbutic principle during and after drying, appears to be a gradual process of spontaneous decomposition, which follows disorganisation of the living cells in association with which it is produced. If the tissues are rapidly brought to an absolutely dry condition, this process of destruction is checked and the anti-scorbutic properties are preserved to a much greater degree (see HOLST and FRÖLICH, 1913). In a similar way these workers found that the expressed juices of acid fruits shewed more stability in respect of their anti-scorbutic value than those of fresh vegetables, and concluded that the rate of this spontaneous decomposition of the vitamine was slowed by the presence of an acid medium. This affords some scientific foundation for the general belief that preserved fruit juices may be relied upon to retain some, at least, of their original anti-scorbutic value.

(B) High Temperatures.—There are many isolated observations upon the influence of exposure to high temperatures upon the anti-beriberi vitamine, but no one hitherto has attempted any systematic study of this point. GRIJNS (1901) found that 1-2 hours' exposure to 120° C. destroyed the protective powers of unmilled rice, Katjangidjo beans *(Phaseolus radiatus)*, and buffalo meat against avian polyneuritis. EIJKMAN (1906) and SCHAUMANN (1910) confirmed GRIJNS' results on the whole, though the former observer found a greater resistance present in the case of horseflesh. HOLST (1907) detected no particular loss of anti-neuritic vitamine after exposure of dried peas and unpeeled barley to 115° C. for half an hour, but some damage in case of beef after half an hour at 110° C. Most observers have failed to find any significant destruction of this vitamine at 100° C.

The discrepancy between the results of these various experiments is to be explained partly by the roughness of the biological test for the presence of the vitamine in question. It is also partly due to the fact that certain substances originally containing a low concentration of this vitamine (*e.g.*, meat) may be unable to protect from or cure polyneuritis after a certain degree of exposure to high temperatures, whereas others originally richer in this material (*e.g.*, pulses, cereals, etc.) may, after a similar exposure, yet retain a sufficient amount to afford satisfactory protection or cure. Another point to be considered in these experiments is that the temperatures noted appear to be those registered by the autoclave or steamer in which the heating was done, and that no measurements are recorded of the temperatures in the interior of the substances heated. This is an important point, as the latter temperatures remain for a long time below the former, the degree of difference depending on the conductivity of the material heated, and will vary with every type of substance investigated. It therefore seemed to us worth while to make a series of systematic experiments on this point, and a brief résumé of the results obtained* is set forth in Table V.

Two substances were chosen for the investigation, both rich in anti-beriberi vitamine, viz. *(a)* the germ *(embryo)* of wheat, and *(b)* yeast Extract A.

TABLE V.

INFLUENCE OF EXPOSURE to high temperatures upon the anti-beriberi vitamine contained (*a*) in Wheat Embryo and (*b*) in Yeast Extract.

SUBSTANCE	Temperature. °C.	Time. Min.	Minimum amount required to cure a pigeon (300 to 400 grms.) suffering from acute Polyneuritis. (Grams).
Wheat embryo—			
Water content, 11 to 14 % ...	unheated	Control	1·0 to 2·5
.. .. ..	98 to 103	120	2·5
.. .. ..	100 to 117	40	5·0
.. .. ..	118 to 124	120	10·0, did not cure
Yeast extract, A—			
Water content, 30 %	unheated	Control	1·5 to 2·0
.. .. ..	100	60	2·0 to 3·0
.. .. ..	122	60	2·5 to 3.0
.. .. ..	122	120	about 5.

The data in columns 2 and 3, as to the temperatures reached and the times of exposure, refer to the internal temperatures of the material heated, and were compiled in accordance with the results of a special set of control experiments made for the purpose. The vitamine content of the different samples was estimated by determining the minimum amount necessary to cure acute polyneuritis of pigeons, brought on by an exclusive diet of polished rice. The results in Table V. shew that the anti-neuritic properties of wheat-germ and yeast extract are only slowly impaired by prolonged heating at or near 100° C., but that destruction is much more rapid at temperatures in the neighbourhood of 120° C.

The experiments at 100° C. were devised to be comparable with the ordinary processes of cooking, while those at temperatures from 110° C. to 120° C. were arranged to throw light upon the probable fate of anti-neuritic (anti-beriberi) vitamines during the sterilisation of tinned foods,

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[*] This work is given in detail with full protocols of the individual experiments in a special communication upon the subject—see *Proc. Roy. Soc.*, *B*. 1917, read February 15th, 1917 (in the press).

such as tinned meat, etc. In this connection it is interesting to compare the curative values given in Table II. of—

(a) Raw beef, where the curative dose was an extract equivalent to 30 grams dry weight of the original, and

(b) "Maconachie" ration and "New Army" ration, where an "incomplete cure" and "no cure" were obtained with extracts equivalent to 106 and 112 grams dry weight, respectively.

The destructive influence of high temperatures upon the anti-beriberi vitamine is also seen by noting the superior curative properties of dried peas (unheated) compared with "kilned" pea flour.*

As was the case in exposure to drying, so in exposure to high temperatures, the anti-scorbutic vitamine appears to be much more unstable than the anti-beriberi vitamine. There exists no systematic experimental work upon this point, and we are now starting a series of experiments with the hope of filling the gap. There are, however, some valuable observations of HOLST and FRÖLICH (1912), all of which point to the great sensitiveness of this vitamine to high temperatures. When cabbage leaves were boiled at 100° C., the results obtained indicate that at least one half its anti-scorbutic value was lost in 30 to 60 minutes, and after heating to 120° C. for 60 minutes almost *all* power of protection against scurvy was destroyed (*loc. cit.* Table IV., B p. 70).

In the light of these results it is not to be expected that the anti-scorbutic vitamine will survive the heating to which tinned foods are subjected in order to render them sterile.

III. PRACTICAL APPLICATION OF THE EXPERIMENTAL WORK TO THE PREVENTION, RESPECTIVELY, OF HUMAN BERIBERI, AND HUMAN SCURVY.

The practical use that can be made of these investigations on the distribution of the two classes of essential vitamines will depend on the identity of avian polyneuritis and human beriberi on the one hand, and of guinea-pig scurvy and human scurvy on the other. As regards etiology, nature of symptoms and method of cure, the analogy in both cases is so perfect that they can be considered as physiological equivalents, and the information thus acquired can be applied forthwith.

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* This fact needs emphasis, since many samples of pea flour on the market are kilned in the process of preparation, although no mention of this fact is made on the wrapper, etc.

There is, however, in addition, a great deal of direct evidence to be extracted from human experience. The individual instances may not seem to be altogether conclusive, but when taken together they form a mass of evidence which is very impressive, and afford a striking confirmation of the results obtained from experimental work. To exhaust these instances would need a very long communication. We propose, however, to describe briefly a few cases which seem to us among the more instructive.

PREVENTION OF BERIBERI.

Importance of the nature of the Cereal food in the prevention of human beriberi.—In almost all cases human beriberi is to be attributed to a defect in character of the cereal employed in the diet.

The best known and best studied case is that of the rice-eating populations of the Dutch Indies, Malay States, the Philippine Islands, etc.: it has been the subject of numerous series of researches, among which those of EIJKMAN, GRIJNS, BRADDON, FRASER and STANTON, VEDDER and CHAMBERLAIN are among the more important. All these workers are agreed that the disease is caused by a defect in the rice taken, which defect can be traced to the complete decortication which takes place in the modern steam-milling of the grain. Beriberi is prevented in cases where unmilled rice is substituted for the "polished" rice, or where the bran ("polishings") removed in the milling is added to it. The general opinion is that the anti-beriberi vitamine is contained in the innost layer of cells *(aleurone layer)* of the skin, which layer of cells is removed with the bran in the process of preparation. Our experiments indicate that the *germ (or embryo) of the grain, also removed during milling, is the principle source of the anti-neuritis vitamine,* present also to a less extent in the bran (see Table II.). It is not so generally realised, however, that the same principle applies to other cereals, or that an equal danger of beriberi is to be apprehended for a wheat-eating population under certain circumstances. In the modern "roller"-milling of wheat, there is complete separation of bran and germ from the flour: unless, therefore, these constituents are purposely included later (as in brown flour, and "standard" flour) one may regard ordinary white flour and the bread or biscuit baked from it, as being free from these valuable constituents

The deficiency of anti-neuritic vitamine in white wheaten flour was shewn by HOLST (1907), EDIE and SIMPSON (1911) and also by ourselves (see footnote page 150), to occasion polyneuritis in pigeons in a manner exactly similar to polished rice. The following three incidents shew that these results are also applicable to the case of beriberi in man. Under ordinary circumstances this deficiency of vitamine in white bread is well supplied in the other articles of the usual mixed diet of the European. It is in cases where extremes of climate restrict the variety of available foodstuffs, or where there is temporary separation from fresh food supplies on long sea voyages, or by the exigencies of active service on long campaigns, that this defect in the bread ration may become apparent in a tragic manner.

(1) LITTLE (1912) states that in Newfoundland and Labrador, where in mid-winter and spring many persons are obliged to subsist largely on bread, beriberi frequently occurs. At the present time the bread is made from fine wheat flour ; in the memory of the older inhabitants, when the bread was made from " brown " flour, the disease was unknown. In 1910 the following interesting event took place :—A ship laden with whole-wheat flour ran ashore, and a considerable proportion of her cargo was removed in order to lighten her, and later was consumed by the adjacent population. There was no case of beriberi in that region for a year following this occurrence.

(2) Beriberi was rare on Norwegian ships before 1894, after which date it became much more frequent. This frequency coincided with an alteration of diet which was made compulsory in that year in response to a popular demand for an " amelioration " of the conditions of life in the Norwegian mercantile marine. Previously the sailors on long voyages used biscuit made from rye flour ; subsequently the masters of ships were obliged to supply bread baked from white wheaten flour, or a mixture of wheat and rye flour (HOLST, 1911). It is an interesting corollary upon our own experimental work to note that *in the milling of rye flour there is no separation of the germ.*

(3) The most impressive case of all is to be found in the tragic experiences of our own troops recently operating in Mesopotamia. In his " Account of the Medical Arrangements, etc., during the Siege of

Kut-al-Amara" (Dec. 1915 to April, 1916),[*] Colonel HEHIR, I.M.S. (1917), states, "in the early stage of the siege a recrudescence of beriberi amonst British troops gave rise to some apprehension, *but it then disappeared;* whilst in Indian troops and followers during the latter half of the siege scurvy caused anxiety." From other entries in this vivid and valuable diary, it is seen that the British troops in the garrison received a cereal ration of wheat flour during the first two months of the siege. After February 5th, 1916, from one-third to one-half of this flour was replaced by barley flour and by "atta," the coarsely-milled wheat usual in the Indian sepoy's ration. It is very significant that beriberi should have broken out among the British troops while upon their normal ration of white wheaten flour, and should have cleared up when they were obliged to share in the more coarsely milled (and doubtless germ-containing) grain of their Indian fellow-soldiers. There is no doubt, from the wealth of detail given in Colonel HEHIR's report, that the British troops were protected from scurvy by the ample rations of meat[†] (12oz. at first; later, Jan. 22nd, 1916, 8oz.), or horseflesh (1¼lbs. March 4th, 1916), served out to them throughout the siege. The Indian soldiers, while protected from beriberi by the nature of their cereal ration, failed, in many cases, to obtain a sufficient supply of anti-scorbutic vitamine, owing to their refusal to eat fresh meat, in spite of the admirable and persuasive manifesto issued by Colonel HEHIR upon the subject.

During the whole operations in Mesopotamia the Indian soldier seems to have been well protected from beriberi. This was to be expected, for, in addition to an unspoilt cereal, he normally gets a generous daily ration of "dhall," consisting of various dry pulses, which, as may be seen from the foregoing work, are also valuable sources of anti-beriberi vitamine. These, however, did not afford protection from scurvy, which was specially prevalent among the Indians, as, for example, in the summer of 1916, when, presumably, great difficulty was experienced in providing fresh fruit and vegetables *(Mesopotamia Report, X. 42).*

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[*] Appendix III. Mesopotamia Commission Report, 1917.

[†] This meat appears to have been fresh, except possibly between dates of Dec. 30th and January 29th. Fresh meat has some protective power against beriberi, but this is slight compared with that of grain and pulses. It would be of great interest to know whether, during the outbreaks of beriberi among British troops, prior to the siege of Kut (Colonel HEHIR speaks of a "recrudescence" during the siege) the men were receiving tinned or fresh meat.

The deduction to be drawn from all this experience is as follows:—

For the prevention of beriberi it is in the highest degree desirable that the germ (embryo) and the bran of wheat should not be excluded from the flour destined for manufacture of bread and biscuit for troops on active service. This is the more necessary when the troops are separated from fresh food supplies and the rest of the ration consists largely of tinned foods, seeing that these articles are deficient in* all *vitamines, owing to their previous sterilisation at high temperatures.*

Yeast and Yeast Extract.—Yeast is acknowledged to be a most valuable source of anti-neuritic vitamine. There is not much evidence at present available as to the value of yeast and yeast extracts in the prevention of human beriberi; we have not been able to find any report of such a trial. Ordinary dried yeast is very disagreeable to take, and causes digestive disturbance, but many preparations possess none of these defects. They have the agreeable savoury taste of a meat extract, and are largely used as an ingredient in certain soup cubes on the market. We have examined one of the principal commercial yeast extracts (Yeast extract A, Tables I. and II.), and could detect no loss of the original vitamine of the yeast owing to the method of preparation. Soup squares form at all times a very popular addition to the soldier's ration, and might well be limited to those containing a certain proportion of yeast extract, seeing that pure meat extracts have been found deficient in anti-beriberi vitamine (see Table II.). By such a measure a valuable extra supply of anti-beriberi vitamine could be added to the ration in a convenient and palatable form.

Dried Eggs. It will be seen from the examination of two commercial samples of desiccated eggs given in Table II., that so valuable an article of diet as the fresh egg can be successfully dried without losing the anti-beriberi properties. Dried eggs may prove too expensive to form any part of the ordinary soldier's or sailor's dietary, but there can be no doubt as to their suitability for inclusion in hospital stores. Intestinal infections form a large part of the medical casualties in armies on active service, especially when operating in hot climates, and, during both the acute stage and the convalescence, the diet is usually restricted to tinned milk and invalid foods, all of which may be regarded as vitamine-free. Mention is made below of the possibility that intestinal affections may in some cases prove a predisposing cause of deficiency disease. It is, therefore, highly desirable that all supply of vitamine should not be cut off during such an illness. Fresh eggs, which provide anti-neuritic vitamine and are also well suited for an invalid diet, are seldom obtainable, and we would, therefore, strongly recommend that dried eggs should be freely used as a substitute. Both commercial samples examined by us were found to be very rich in anti-beriberi vitamine, they were soluble and could be taken raw, and, when cooked, they made excellent and very palatable dishes.

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* In packing and transporting whole-meal or germ-containing flour, extra precautions are usually considered necessary to protect from damp, and the advice of experts should be sought upon these points. No possible difficulty could arise in the case of biscuit baked before transport. Certain samples have been specially investigated by us and were found to be entirely satisfactory for the prevention of beriberi.

PREVENTION OF SCURVY.

It is a platitude that scurvy can be prevented by the inclusion of fresh vegetables and fruit in a dietary. These are frequently impossible to obtain under the conditions of active service. It is, therefore, our intention under this heading to treat only of such substitutes as are convenient for transport, and hence suited to the needs of armies in the field.

Germinated Pulses as a Substitute for Fresh Vegetables.—The most important fact that has emerged from the study of experimental scurvy is the discovery of Fürst (1912) that the anti-scorbutic vitamine, absent or deficient in the resting seed, makes its appearance in the early stages of germination. In our own experiments with peas and lentils, these were first soaked in water for 24 hours, the temperature ranging from 50° to 60° F.; at this stage a small amount of anti-scorbutic vitamine could already be detected. After germination for a further 48 hours, with access of air, the vitamine-content had increased five- to six-fold, and at this stage the germinated material may be considered the equal of fresh vegetables as regards its anti-scorbutic value (see Table IV. above).

It is difficult to imagine any circumstances in which this form of anti-scorbutic food could not be made available. In the dry form, peas, beans, lentils and other pulses contain only 10 to 15 per cent. of water, and are eminently suited for transport. They are commonly included in the active service dietaries of armies, and in the case of Indian troops, as "dhall," form a considerable proportion of the daily ration. The only change needed is that these pulses should be issued in the unmilled (not husked or split) condition, and should retain the original seed-coat. Then, in case of unavoidable shortage of fresh fruit or vegetables, a substitute could be made immediately available on the spot by germinating the pulses included in the stores.

The whole operation consists of a preliminary soaking, in which the seeds absorb over 100 per cent. of their original weight of water, followed by germination ; after this the food should be cooked and eaten as soon as possible. The time taken by the complete operation could be reduced to 36 hours in a hot climate. In Appendix I. below, p. 178, a set of directions is given, which may be found a useful guide for preparing these germinated pulses in the field.

It is very important that the peas, lentils, etc., should be cooked and eaten as soon as possible after germination, and should not be allowed to dry again. In the process of drying, the anti-scorbutic vitamine developing during the germination, will be destroyed. Having regard to the sensitiveness of this vitamine to high temperatures, it is also important that the pulses should not be cooked longer than is necessary to render them soft and palatable. A period not exceeding 1 to 1½ hours should suffice for peas and about half an hour for lentils. No deterioration in flavour can be detected after germination has taken place.

Dry pulses contain a high proportion of anti-beriberi vitamine, and this was found by GRIJNS (1901) to be lessened in amount as germination takes place.

We found that in the *early* stages of germination an abundant supply of anti-beriberi vitamine was still retained by our peas and lentils. Dramatic cures of pigeons with acute polyneuritis were obtained with moderate doses (5 to 10 grams) of peas and lentils germinated to the stage, shewn in Fig. 6, in which condition we were using them successfully for the prevention of scurvy.

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* Captain COOK, whose long voyage (1772-1775) was notable for the continued good health of the sailors, always took with him a large supply of malted barley. He had the greatest belief in the anti-scorbutic value of a freshly made infusion (sweetwort) and served it out to his men in case of need ("Captain Cook's Voyages," *Everyman* Edition, p. 227).

Some Chinese are accustomed to take part of their daily rice in the germinated condition, and this custom has spread to the Malay States (private communication from Brig.-Gen. ANDERSON) and Dutch Indies (GRIJNS, 1901), where "towgay" or germinated pulses are regularly taken. There appears to be no evidence, however, that the anti-scorbutic properties of these foods have been appreciated.

Freshly germinated pulses (or cereals),[*] therefore, occupy a special position among foodstuffs in being richly endowed with both the anti-scurvy and the anti-beriberi vitamine.

Vegetables.—Among vegetables the onion is marked out as being specially suited to the need of troops, owing to its great resistance to adverse conditions during transport and to the length of time it will remain wholesome. The anti-scorbutic value is comparable with that of fresh cabbage and its flavour gives it more value as a culinary adjunct than is possessed by any other vegetable

Potatoes were found to be somewhat inferior in anti-scorbutic properties to cabbage, onions, etc. They are more suitable for transport and keep better than most vegetables, and there is no doubt that they have been proved of great value for the prevention of human scurvy. It must be remembered, however, that the amounts consumed are rather large. In one Irish workhouse[†] the daily ration of potatoes is 3 lbs.; after a careful study of convict diets, Dr. GUY[‡] concluded that 14oz. daily would protect from scurvy, the rest of the ration including 1oz. of other fresh vegetable and 4oz. of meat. Outbreaks of scurvy have repeatedly followed failure of the potato harvest in countries where potatoes are a staple article of diet, *e.g.*, Norway in 1904 (HOLST, FRÖLICH, 1912) and Ireland in 1847 (CURRAN). The recent appearance of scurvy in Glasgow[§] and Newcastle and Manchester is no doubt to be attributed to the great scarcity of potatoes during the last three or four months.

Fresh Meat.—The expressed juices of fresh meat gave disappointing results in the prevention of guinea-pig scurvy, but there is no doubt that human beings can be protected by its regular use. This is one of the cases which suggest that the guinea-pig is much more susceptible to scurvy than is man, an opinion also held by HOLST and FRÖLICH (1912). Many clinicians believe fresh meat juice to be capable of curing infantile scurvy, and in many Arctic expeditions where scurvy has been escaped, fresh meat of various kinds has been the only anti-scorbutic material present in the diet.

[*] Refers to note on previous page.

[†] Limavady Union, Co. Derry.

[‡] Dr. GUY'S evidence, Report of the Lords Commissioners of the Admiralty on the Outbreak of Scurvy in the recent Arctic Expedition. 1877, par. 5323.

[§] *Lancet*, July 8th, 1917.

HARLAN. *British Medical Journal.* July 14, 1917.

The following incidents are related by Jackson and Harley (1900:—Six Russian priests spent the winter in a hut at Kharborova, Yugor Straits, with a small Russian boy to wait upon them. Their religion would not permit them to eat reindeer or such meat; they subsisted largely on salted fish, and there were no vegetables. In the following May the little Russian boy was found to be the only surviving person in Kharborava; he was subject to no religious restriction and ate reindeer meat during the winter. Jackson himself, living among the Samoyads in Waigatz, 1893-1894, noted that among those of the population who winter on the island, and get neither vegetables nor lime-juice, but live largely on fresh reindeer meat, scurvy is unknown. Some Samoyads, however, migrate with Russian peasant traders, and spend the winter near the large rivers in north-east Russia where the diet consists largely of salt fish; among these scurvy is prevalent.

There is no doubt, however, that animal tissues are distinctly inferior in anti-scorbutic properties to those of fresh fruit or vegetables, and that a large and regular ration is necessary for safety. This opinion was expressed by many witnesses in the Admiralty Enquiry[*] upon the outbreak of scurvy in the Arctic Expedition of 1875, and in the older literature many instances occur in which an apparently liberal meat ration did not prevent scurvy. Curran (1847) describes three cases admitted to Swift's Hospital, Dublin, in the great Irish epidemic of 1847, where the previous diet had included ¾lb. on five days of the week. Colonel Hehir[†], in a report suggesting needed reforms in the Indians soldiers' diet in Mesopotamia as early as April, 1915, expresses great doubt whether the authorised meat ration of 28oz. weekly is sufficient to prevent scurvy unless more meat is added and the vegetable ration (2oz. potatoes) is increased. If dry weight is taken into account in comparing the anti-scorbutic value of meat and vegetables, 4oz. meat must be regarded as the equivalent of at least 10oz. vegetables. The British troops during the siege of Kut-al-Amara were doubtless protected from scurvy by their meat or horseflesh rations, but these were very abundant (8oz. to 20oz. daily), see above, p. 165.

Milk.—Fresh cow's milk was found to possess very low anti-scorbutic value in feeding guinea-pigs; satisfactory protection from scurvy was only attained by a large ration, about 100 c.c. daily. It is probable that infants living upon cow's milk do not receive any great excess of vitamine. As regards adults, Curran (1847) instances upwards of 80 cases admitted to the Dublin Union Hospitals in the Irish epidemic of 1847; all had been inmates of the union for at least six months previously, and the diet, though deficient in vegetables and meat, had included at least one pint of milk daily.

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[*] "Report of the Lords Commissioners of the Admiralty upon the Outbreak of Scurvy in the Recent Arctic Expedition." London, 1877.

[†] Mesopotamia Report, 1917. X. 41.

We have, nevertheless, been much impressed with the great value of milk, even when heated to 120° C. for one hour to destroy the vitamines, as an adjunct to diet. The general condition of animals who received a minimum amount of anti-scorbutic material could be enormously improved by the addition of a daily ration of this heated milk, although the scurvy was not influenced. Therefore, while admitting that milk is of little importance for the prevention of scurvy (or beriberi), its inclusion in a diet, even when tinned and sterilised, would appear to be a very valuable measure.

Fruit.—Fresh fruit juices appear to be among the most valuable anti-scorbutic materials we possess. In our experimental work fresh orange and lemon juices were found very potent. There is also abundant evidence, dating as far back as the beginning of the seventeenth century,[*] of their value in the prevention of human scurvy. In 1795, largely due to the efforts of Sir Gilbert Blane (1785, 1830), a regular issue of lemon juice[†] was ordered in the Navy, and a remarkable decrease of the death-rate was the result. Budd (1840) states that, whereas 1,457 cases of scurvy were admitted to the Royal Naval Hospital at Haslar in the year 1780, no case was reported in the years 1803 to 1810.

The juice of limes has also been widely esteemed in the past as an anti-scorbutic. There is not, however, much trustworthy evidence as to the anti-scorbutic value of lime-juice when preserved according to modern methods. We have so far found it disappointing for the prevention or cure of guinea-pig scurvy. Holst and Frölich (1912, p. 96) on the other hand, demonstrated distinct anti-scorbutic properties in two commercial samples of lime-juice, purchased in retail shops in Christiania. The subject is one of very great importance, and we are at present engaged on a further investigation, with special reference to the processes of preservation. In the meantime we are not able to express any definite opinion. One thing, however, seems very clear. There is no reason at all why preserved lime-juice should be regarded as a specially concentrated form of anti-scorbutic. This has seemed to be the view generally held, if one may judge by the small size of the ration issued. If lime-juice is issued at all as an anti-scorbutic the ration should be a liberal one—at least 1oz. daily.

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[*] James Lind. "A Treatise on Scurvy," 2nd Ed. London, 1757.

[†] In 1840, the daily ration of lemon juice was 1oz. and 1½oz. sugar, issued two weeks after leaving land. (Budd, 1840).

IV. RELATION BETWEEN BERIBERI AND SCURVY AS REGARDS TIME OF ONSET, ETC.

It is evident that a diet defective in both classes of vitamine will occasion both beriberi and scurvy, but it is likely that beriberi will precede scurvy in point of time. In "ship" beriberi the symptoms are reminiscent of scurvy, and it is probable that there has been in the diet a deficiency both of anti-beriberi and anti-scurvy vitamines. The "period of resistance," or "period of development," will depend on the degree of deficiency and upon the idiosyncracy of the individual, but, on the whole, seems to be shorter in case of beriberi. There is good evidence upon this point in the observations of FRASER and STANTON (1909) who found beriberi developing among Javanese coolies after about 80 to 90 days upon a diet composed largely of polished rice. In case of scurvy, the time seems to be longer. Colonel HEHIR* considers four months to be the minimum time in which scurvy will appear in Indian troops on active service, and HOLST and FRÖLICH (1912) refer to two instances in which the time was longer. The first is that of a fanatical vegetarian who wished to prove to the world that human life could be sustained upon a diet of bread and water. The bread was presumably rye bread, and the individual therefore well supplied with anti-beriberi vitamine; he developed scurvy in 7½ months. The second was related by a political refugee in Norway, and deals with the case of 1,400 prisoners in a Russian convict prison. The diet consisted of tea, coarse bread and cabbage soup; the preparation of the latter was so unclean and disgusting that twenty of the prisoners, who were of gentler birth than the others, could not endure to take it. After about six months these twenty shewed symptoms of scurvy, while no case occurred among the rest of the prisoners who took the soup. This incident also shews that, even after a prolonged boiling, enough anti-scorbutic vitamine remained undestroyed in the cabbage leaves to protect from scurvy.

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* Mesopotamia Report, 1917, X., 41.

It is not improbable that the times of onset of these two deficiency diseases might be much shortened in case of any intestinal infection occurring during the "period of development." Such a disease might well impose a drain upon the metabolism which would involve the reserve of vitamines as well as the other resources. It is interesting in this connection to note that among twenty-six cases of beriberi diagnosed in our troops in the Dardanelles in the autumn of 1915, and noted by WILLCOX (1916), eighteen had suffered from diseases of the digestive and alimentary system, previous to the beriberi. At the same time it is possible that the first illness may only indirectly have predisposed to the beriberi by making it necessary to further restrict the already limited diet to such vitamine-free articles as tinned and sterilised milk, etc.

The following case (for details of which I am indebted to Dr. FRANK SAVERY, of Ealing) of a little girl, aged two, who acquired scurvy while suffering from chronic colitis is also to the point. Cow's milk could not be taken and the child was nourished on a brand of dried milk, with the addition of raw meat-juice to her diet as an anti-scorbutic. After some months on this diet, scurvy was diagnosed, but was readily cured by fresh orange juice. Fresh meat-juice has been considered by many clinicians an adequate anti-scorbutic for infants upon a diet of sterilised milk, and is also much esteemed as a cure for infantile scurvy. This incident suggests that the colitis rendered the child more susceptible to scurvy than a normal infant, and also points to the great anti-scorbutic potency of fresh orange juice in comparison with meat-juice.

SUMMARY.

1. To maintain a human being in a satisfactory state of nutrition the diet must contain—

(*a*) A suitably proportioned supply of protein, fat, carbohydrate, salts and water, and

(*b*) An adequate amount of accessory food factors, or vitamine.

Both (*a*) and (*b*) are required; excess of one cannot make good any deficiency in the other.

2. These necessary vitamines are, at least, of two kinds: firstly, the anti-neuritic or anti-beriberi vitamine, deficiency of which in a diet occa-

sions beriberi; and, secondly, the anti-scorbutic vitamine, deficiency of which occasions scurvy.

3. Neither of these vitamines has yet been isolated in a pure state, and, for the moment, their presence can only be detected by biological methods.

4. These two classes of vitamines have each their individual rôle in metabolism; they possess properties different from each other and are differently distributed among natural foodstuffs.

5. The distribution of the anti-beriberi vitamine has been investigated by study of experimental polyneuritis in birds, which is generally accepted as analogous to human beriberi. Pigeons, if deprived of anti-beriberi vitamine (*e.g.*, on an exclusive diet of polished rice or white flour, etc.), develop acute polyneuritis (beriberi) in fifteen to twenty-five days. The presence and relative amount of the anti-neuritic vitamine contained in various foodstuffs has been determined by means of curative experiments, and by preventive trials with specially selected diets. In this work we have extended the observations of COOPER (1913-14), and have, in general, followed his methods.

6. The anti-beriberi or anti-neuritic vitamine was found in almost every natural foodstuff examined. The principal source is in the seeds of plants, *e.g.*, cereals and pulses. The most important result emerging from the present work is the fact that *in cereals the anti-beriberi vitamine is mainly deposited in the germ or embryo of the grain, and to a less extent in the bran.* White wheaten flour or polished rice, which consist of the *endosperm* (minus *aleurone layer*) of the grain are deficient in this vitamine, and if employed as sole diet will occasion polyneuritis in pigeons or beriberi in man.

Other important sources of anti-neuritic vitamine are the eggs of animals (*e.g.*, hens' eggs or fish roe) and yeast or yeast extract. Milk and cheese gave disappointing results (COOPER, 1914).

7. From the abundant presence of the anti-beriberi vitamine in dry foodstuffs it is clear that this substance is resistant to drying. It can also withstand exposure to temperatures in the neighbourhood of 100° C. for two hours without significant loss; if heated under pressure to, or near, 120° C. destruction takes place more rapidly (see Table V.).

8. The distribution of the anti-scorbutic vitamine has been investigated by a study of experimental scurvy in guinea-pigs. We have

followed the methods of HOLST and his co-workers (1912) with some modifications. A diet of cereals with water or sterilised milk will cause death from acute scurvy in these animals within a month. The influence of various foodstuffs in preventing scurvy when added to this "scurvy diet" has indicated where the principal sources of the anti-scorbutic vitamine are to be found.

9. The anti-scorbutic vitamine is present in active living vegetable tissues. It is also present in animal tissue, to a much less degree. Fresh vegetables and fresh fruit juices are the most valuable sources of anti-scorbutic vitamine that we possess. All the dried foodstuffs examined, including desiccated vegetables, were more or less deficient in this vitamine.

10. Dry pulses (or cereals) though rich in anti-beriberi vitamine are deficient in anti-scorbutic vitamine, and afford no protection against scurvy. If these are moistened, however, and allowed to germinate, the anti-scorbutic principle is regenerated with the beginnings of active cell life.

Germinated pulses are recommended as a valuable and convenient means of preventing scurvy in the absence of fresh fruit and vegetables. In the dry stage they are eminently suitable for transport and can be moistened and germinated on the spot as required. In Appendix I. is given a method for preparing germinated lentils or peas under active conditions.

11. It is evident from 9 and 10 that the anti-scorbutic vitamine is extremely sensitive to drying. As regards exposure to high temperatures, it is also more unstable than the anti-beriberi vitamine. HOLST (1912) found that the anti-scorbutic power of fresh cabbage was slightly lessened after exposure to 100° C. for half an hour, but that the deterioration was appreciable after one hour; at 110-120° C. the destruction was rapid and complete.

12. It follows from 7 and 11 that neither the anti-neuritic vitamine nor the anti-scorbutic vitamine may be expected to survive in tinned or sterilised foods, considering the high temperature to which these have been subjected in course of preparation.

13. In case of armies or other populations subsisting largely on tinned food, it is imperative to provide adequate supply of vitamine from outside sources.

To prevent beriberi, the bread or biscuit should be made from wholemeal or germ-containing flour.

To prevent scurvy, if a supply of fresh fruit or vegetables is not procurable, germinated pulses should be added to the diet.

In conclusion, our best thanks are due to the following firms for generous help in the supply of material for our investigations to:—Messrs. Steele & Co., rice millers; Messrs. Brown & Polson, maize millers; The Hovis-Bread Flour Co. and Messrs. J. & H. Robinson, wheat millers, and especially to Mr. E. G. Ellis, of the last-named firm for much kind help, information and advice; Messrs. L. Rose & Co., for samples of specially prepared lime-juice; Elizabeth Lazenby & Son, Harrod's Stores, and Messrs. Crosse & Blackwell. We also wish to express our thanks to Professor HARDEN, F.R.S., for preparing some of the extracts mentioned in Table II.; to Miss MABEL RHODES, for kindly making the drawings from which figures 1 to 6 are made; and lastly to our assistant, Mr. A. H. ROBINS, for his devoted care and feeding of the experimental birds in the beriberi enquiry.

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APPENDIX I

SUGGESTED METHOD OF USING PEAS, LENTILS, OR OTHER PULSES (DHALL) FOR THE PREVENTION OF SCURVY, IN THE ABSENCE OF FRESH VEGETABLES.

1. The dry seeds must be whole, retaining the original seed-coat, not milled or decorticated. See Fig. 5 (*a*) peas and (*b*) lentils.

2. They must be soaked in water for several hours; the time necessary depends on the temperature, 24 hours at 50° to 60° F. and 12 hours or less at 90° F.

3. The water must then be drained away and the peas, etc., allowed to remain in the moist condition with access of air. They will then germinate and the small rootlet grow out as in Fig. 6 (*a*) peas and (*b*) lentils. This germination will take 48 hours at 50° to 60° F. and 12 to 24 hours at 80° F.

4. The operations described in 2 and 3 could conveniently be done under active service conditions in such manner as the following :—

> *Soaking.*—The peas or other pulses, placed in a *clean* sack, should be steeped in a trough, barrel, or other suitable vessel, full of clean water, and should be occasionally stirred. The sack and trough, etc., should be large enough to allow for the swelling of the peas to about three times their original size. In a hot climate 6 to 12 hours should suffice for this soaking.
>
> *Germination.*—The peas should be lifted out of the water and spread out to a *depth not exceeding 2 to 3 inches* in a trough or other vessel with sides and bottom porous or well perforated with holes. This is to allow *complete* access of air. *The seeds must be kept in a moist atmosphere,* This is done by covering with damp cloth or sacking, which is sprinkled (by hand or automatically) as often as is required to keep the peas thoroughly moist underneath. The germinaiion should reach the stage shewn in Fig. 6 within 24 hours in a hot climate. *All the vessels should be clean.*

5. *It is important that the germinated pulses should be cooked and eaten as soon as possible after germination, and should not be allowed to become dry again;* in that case the anti-scorbutic properties, acquired during the process of germination, will again be destroyed. The pulses should not be cooked longer than necessary.

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An Estimate
OF THE
ANTISCORBUTIC VALUE OF MILK IN INFANT FEEDING

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An Estimate

OF THE

ANTISCORBUTIC VALUE OF MILK IN INFANT FEEDING.

SCURVY, adult or infantile, may now be classed definitely with beri-beri among the so-called deficiency diseases. Both general experience with human beings and modern experimental work upon animals have shown that these maladies arise as the result of a long period, usually several months, of imperfect nutrition. The imperfection is referred to absence or deficiency in the diet of certain unknown substances whose presence is essential for satisfactory nutrition.

Some recent work (e.g., Cooper, 1913 and 1914; Holst and Fröhlich, 1907 and 1912; H. Chick and E. M. Hume, 1917) has shown that the antiscorbutic and antineuritic vitamines, the accessory food factors, whose absence from a diet occasions respectively scurvy and beri-beri, differ from each other in their distribution among foodstuffs, in their properties, and in the rôle they play in metabolism.

The anti-scurvy factor is found to be present in living, actively metabolising tissues of plants and (to a less degree) of animals, whereas the chief sources of the anti-beri-beri factor are the seeds of plants and the eggs of animals. The most valuable antiscorbutic materials we possess are green leaves (such as cabbages and salads) and fresh fruit juices; onions and the root vegetables, potatoes, carrots, swedes, &c., come next in importance; lastly, there are fresh meat and milk.

The anti-scurvy vitamine suffers a spontaneous decomposition when the substances containing it are preserved by drying or any other means. This destruction takes place slowly at ordinary or low temperatures, but occurs much more rapidly at high temperatures, the rate depending on the actual temperature employed. Some loss is therefore to be expected in cooked vegetables, fruit, or milk, depending on the temperature and time of cooking.

Results of Present Investigation.

As regards the possession of anti-scurvy properties, milk must be classed among the less valuable materials. If reliance is to be placed upon its presence in a diet as the only means of preventing scurvy, the amounts consumed must be large, and any methods of preparation, such as heating or drying, which involves diminution of the small amount of antiscorbutic substance originally contained, must be regarded with suspicion. This is clearly shown in the results of experimental work set forth below and confirmatory evidence is also to be found in experience of human diets.

The results of our experimental work on milk are summarised in the table, and full details will be found in a paper shortly to appear in the *Biochemical Journal.* Following the methods of Holst, young growing guinea-pigs of about 200-400 g. were selected as the experimental animals. The greenstuff was withdrawn from the normal diet of oats, wheaten bran, and cabbage leaves, and raw cow's milk given instead.

On a diet of oats and bran *ad libitum*, with the addition, of course, of water, young guinea-pigs fail to grow, although the initial weight is maintained until about the eighteenth day of the experiment. (See Experiments 1, 2, and 3.) About this period tenderness of the limbs and joints can be detected, and the animal assumes various characteristic and unusual positions, with the evident intention of sparing the painful member. In a certain proportion of cases the jaw or face becomes sore and the animal obtains relief by lying with the side of the face resting on the floor of the cage. At the same time a fall in weight begins, the incisor teeth become loose (it is impossible to examine molar teeth in a guinea-pig during life), and little solid food is taken. The animal will usually die about the twenty-fifth to thirtieth day.

The post-mortem appearances show close analogy with those of human scurvy, closer perhaps with those of infantile scurvy. Subcutaneous and intramuscular and periosteal hæmorrhages are found, especially in the limbs, the sternum, and ribs. Hæmorrhages may also be present in the intestines or in the muscles of the heart; in these cases death usually occurs suddenly and at an earlier date.

When the water in this "scurvy" diet was replaced with cow's milk, it was found that a large daily ration was required to prevent the onset of scurvy. It was, in fact, necessary that the diet should become practically a milk diet. Short of this, we were unable to prevent scurvy, although the onset of symptoms was delayed and growth of the animal maintained in proportion to the quantity of milk consumed daily. It was found, for example, that when 30–50 c.cm. of raw cow's milk was taken daily (Experiments 4 and 5) death from scurvy ensued in about 30 days, a time not much longer than in Experiments 1 to 3, where water was given instead of milk. When 50 c.cm. was consumed daily throughout and this amount maintained by careful hand-feeding, the onset of scurvy was delayed and the life of the animal prolonged to 75 days. In Experiment 8, animal No. 242 took a daily average of 86 c.cm. at first, and later 60 c.cm.; the length of life was 90 days, and scurvy symptoms were noticed first on the forty-fifth day. Finally, when the

Antiscorbutic Value of Cow's Milk.

No. of experiment.	No. of animal.	Diet.	Milk ration, average amount consumed daily, c.cm.	Weight of animal, grammes.				Length of experiment, days.	Day of experiment, on which scurvy symptoms were first noted.	Result.
				At beginning.	Highest reached.	At end.	Change of weight during experiment, %.			
1	46	Oats, bran *ad lib.**	0 (water given)	325	330	215	−34	25	18	Death from scurvy, 25th day.
2	13	,, ,,		340	340	200	−41	21	13	,, ,, 21st ,,
3	47	,, ,,	—	280	305	185	−34	30	18	,, ,, 30th ,,
					Raw Milk.					
4	43	,, ,,	30	285	340	215	−25	31	20	Very ill, scurvy; killed 31st day.
5	61	,, ,,	50 for 14 days, afterwards 36.	266	310	220	−15	30	15	Death from scurvy, 30th day.
6	236	,, ,,	50	451	469	240	−47	75	33	Slow chronic scurvy from 33rd day. Killed 75th day.
7	234	Oats, bran, 10 g.	64	275	340	268	−3	83	21	Death from scurvy, 83rd day.
8	242	,, ,,	86 for 44 days; afterwards 60 c.c.	453	490	300	−34	90	45	Slow chronic scurvy from 45th day. Death from scurvy, 90th day.
9	235	,, ,,	85 for 22 days, afterwards 130 c.c.	260	Inc. to 585		+125	100	—	Good health throughout.
					Dried Milk.†					
10	163	Oats, bran *ad lib.*	60	346	373	237	−32	34	28	Death from scurvy, 34th day.
11	162	,, ,,	76	350	395	350	—	23	21	Cure attempted, 23rd day.
					Autoclaved Milk. 120° C. 1 hour.					
12	173	Oats, bran *ad lib.*	55	405	500	298	−26	40	21	Very ill, scurvy, killed 40th day.
13	176	,, ,,	41	341	382	245	−28	39	21	Death from scurvy, 39th day.
14	49	,, ,,	50	290	360	230	−21	28	22	Death from scurvy, 28th day.
15	239	Ditto + 3 c.cm. fresh orange juice	ca 60	365	Inc. to 598		+64	93	—	Good health.
16	440	,, + 2·5 g. cabbage.	ca 60	320	,,	572	+79	95	—	,,
17	423	,, + 5 g ,,	ca 60	299	,,	565	+89	92	—	,,
18	103	,, + 10 c.cm. raw beef juice.	0	350	350	240	−31	23	15	Death from scurvy, 23rd day.
19	Control.‡	Oats, bran, fresh cabbage *ad lib.*	0	350	Inc. to 724		+107	84	—	Good health, normal animal.

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* In health the animals consumed 30-50 g. daily, in exceptional cases as much as 80-100 g.; when sickening with scurvy the amount taken decreased to about 10-20 g. † Expressed in equivalent of fresh milk. ‡ Mean of 14 observations. Inc. = increasing.

amount of milk consumed, and varied from an average of 85 c.cm. at the beginning, to 150 c.cm. for the main period of the experiment, good health was maintained throughout and the increase in weight approximated to the normal during the three months of the experiment.

The conclusion we draw from these experiments is that cow's milk, when compared with some other foodstuffs (see Experiments 15–17), is a material poor in antiscorbutic properties, necessitating its use in large quantities, in fact as a sole diet, if scurvy is to be prevented by its agency alone. Such a view is in accord with the particular rôle mammalian milk is destined to play in nature—namely, to afford a complete food for nourishment of the species during a considerable, but definitely limited, time after birth.

Bearing of Results on Feeding of Infants

Milk is intended for consumption in the raw condition, and seeing that cow's milk at least is endowed with no great excess of anti-scurvy vitamine, it is necessary to beware of introducing in its preparation any methods which might lead to a diminution in this valuable constituent. Such processes include the heating (pasteurisation or boiling) of milk, adopted of necessity to afford protection from milk-borne disease, and the drying of milk which, in addition, has the great virtue of economy. Experiments 12–14 above show an almost complete loss of antiscorbutic properties to have taken place in both dried and strongly heated (120° C. for one hour) milk. In practice, where milk is heated to a less degree—e.g., "pasteurised" or boiled—loss in its vitamine value is also to be apprehended. When any of these methods are employed in preparing cow's milk for infants it is therefore necessary that the diet should be reinforced with an extra supply of antiscorbutic material from outside.

The freshly expressed juice of oranges and grapes has long been advocated by certain clinicians as a necessary adjunct to the diet of infants when artificially fed. The scientific basis for this opinion is readily seen from the results of Experiment 15, in which the great antiscorbutic value of fresh orange juice is demonstrated. Raw-meat juice has also been employed for a similar purpose by some physicians, but by others is considered inferior to fresh fruit juice, a conclusion which is also in accord with our experimental results (see Experiment 18 with meat juice).

Some infant specialists,[1] however, are inclined to deny the necessity for any extra antiscorbutic in artificial feeding of babies on the ground that infantile scurvy is a rare disease in recent days, notwithstanding the almost universal practice of heating cow's milk before feeding to infants, and the great increase in the use of dried milk. It is maintained that both heated and dried milk have been used extensively for infant feeding, with no addition of antiscorbutic and without any untoward results.

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[1] For a discussion on this subject see Lane-Claypon, "Milk and its Hygienic Relations." 1916.

These authorities should be asked gravely to reconsider their opinion, in the light of the following observations. In the first place, scurvy in the human subject is a disease of very slow development, in which symptoms are manifest only after several months of imperfect nutrition. These symptoms may show every gradation from severe to almost imperceptible changes, according as the deficiency has been complete or slight. There is no doubt that infants are less susceptible to this disease than guinea-pigs, and it is possible that scurvy of a mild latent type is much more prevalent among infants than is supposed, and frequently passes unrecognised and undiagnosed. Special attention was called to this point in an editorial article in THE LANCET of Sept. 22nd last describing the work of certain American observers. Hess and Fish (1914) took advantage of an exceptional opportunity in the Hebrew Infant Asylum, New York, to make an exhaustive study of this condition. It had been the custom to feed the babies upon cow's milk, pasteurised at 145° F. (63° C.) for 30 minutes, and to add fresh orange juice to the diet as antiscorbutic. After publication of the Report of the American Commission on Milk Standards orange juice was omitted from the dietary, as it appeared unnecessary to continue it, in the light of the pronouncement given in this report as to the equal nutritive value of raw and heated milk. The result was that an outbreak of mild scurvy took place in the asylum about three months later. The symptoms were for the most part only slight, the babies displayed fretfulness, marked pallor, loss of appetite, general ill-health, cessation of gain in weight and of growth in length. In the more severe cases hæmorrhages were noticed, also anæmia and distinct tenderness of the joints. All the infants affected were over six months old, and it would have been natural to attribute the indisposition to teething troubles. The scorbutic nature of the disorder was, however, made apparent by the rapid cure of all symptoms when orange juice or other antiscorbutic was restored to the diet, or when raw milk was substituted for the pasteurised milk.

A further contribution to this subject is made by Miller (1917), who has recently given an interesting description of four cases encountered in private practice. None of the cases showed the classical picture of acute scurvy; the children were ailing and fretful and other diagnoses were suggested. In all cases they made rapid recovery after the diet was enriched with antiscorbutic material.

At the present time the regular use of fresh orange juice or fresh grape juice in the infants' dietary may present some difficulty owing to the restrictions placed upon the importation of fresh fruit by war conditions. These fruits are likely to be scarce during the coming winter, and out of the reach of poor households. We are, therefore, at present engaged in determining the antiscorbutic value of raw juices expressed from various root vegetables which are home grown and plentiful, in the hope of discovering a satisfactory and cheap substitute for these fruits. Some encouragement for this line of research already exists in the clinical data available as to the distinct value of potatoes, even when cooked, for the cure of infantile scurvy.

Summary.—1. Raw cow's milk contains the accessory food factor (vitamine) which protects from scurvy, but this is present in small amounts and is further diminished by heating or drying.

2. Infants fed on heated or dried milk, or on any milk substitute, should receive also some antiscorbutic ration, such as fresh fruit juice or potato. It is probable that the raw juices of turnips and swedes can also be employed.

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XVII. THE ALLEGED ANTINEURITIC PROPERTIES OF α-HYDROXYPYRIDINE AND ADENINE.

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(*Received June 5th, 1917.*)

In the hope of obtaining a compound which would possess antineuritic properties Williams [1916] prepared and tested a series of substances. By adopting this line of research he hoped to throw some light on the chemical identity of the active principle which cures polyneuritis in birds and beri-beri in man. He confined himself to the study of pyridine derivatives mainly because the active fraction obtained from rice polishings is closely associated with nicotinic acid and under certain treatment gives off a pyridine-like odour, whilst the colour reactions given by antineuritic substances with phosphotungstic and phosphomolybdic acids respectively are also characteristic of the hydroxypyridines. He prepared and tested a large series of pyridine derivatives including nicotinic, cinchomeric, quinolinic, 6-hydroxynicotinic and citrazinic acids, glutazine, α-hydroxypyridine, 2 : 4 : 6-trihydroxypyridine (and its anhydride) and 2 : 3 : 4-trihydroxypyridine. The last three of the above Williams found to be definitely active but the activity disappeared after some time. As there was no evidence of decomposition, Williams ascribed the loss of the antineuritic properties to isomerisation. To substantiate his hypothesis he made a detailed study of one of the active substances—α-hydroxypyridine. He found that there were two crystalline modifications of this substance, needles and granules. By treating polyneuritic birds with the needle variety Williams managed to effect in every case a definite cure or improvement; the granules on the other hand were inactive. The absolute inactivity of the granular modification of α-hydroxypyridine induced him to put forward the further suggestion that the polyneuritic condition is brought about not by the lack of a particular isomer, since isomers are in equilibrium in solution and any biological preference for one would disturb the equilibrium and thus effect the corresponding conversion, but by a want of a certain type of potential energy supplied by the process of isomerisation of certain substances. In a subsequent investigation Williams in collaboration with Seidell [1916] isolated from yeast an active substance which they considered to be identical with adenine and which lost its antineuritic activity on keeping. This loss of therapeutic potency the authors also ascribed to isomerisation.

If isomerisation is really responsible for the therapeutic activity of antineuritic principles it is of great scientific importance and interest and it was our object to study the matter in detail. We chose α-hydroxypyridine, with which Williams claims to have achieved some success, as our starting point. Although we could confirm most of the facts about the chemistry of that substance we were unable by its use to effect anything which in our opinion could be considered as a cure or even an improvement in the condition of the polyneuritic birds. We have taken great care in following Williams' method in the preparation of the active needles and no doubt exists in our minds that our preparations were identical with those described by Williams. We have also taken great pains in watching our birds and nothing of clinical importance could have missed our notice.

Experimental.

Like Williams we prepared the α-hydroxypyridine by distilling hydroxynicotinic acid according to the method of Pechmann and Baltzer [1891]. The latter was made from the methyl ester of coumalic acid by the method of Pechmann and Welsh [1884]. The coumalic acid was obtained from malic acid by the method described by Pechmann [1891]. Before distillation the hydroxynicotinic acid was decolorised with charcoal and purified by recrystallising it several times from hot 50 % acetic acid. It always gave the characteristic reactions and correct melting point. The α-hydroxypyridine obtained by distilling the hydroxynicotinic acid gave the correct nitrogen figures on determination by the Dumas method.

When hydroxynicotinic acid is distilled the α-hydroxypyridine comes over as syrup which on cooling crystallises in a mixture of needles and nonacicular crystals which Williams classified as granules. In Williams' opinion the amount of moisture present in the process of distillation is a determining factor as to the relative amounts of the two crystalline modifications formed. We found that by excluding moisture entirely needles only were formed.

We therefore heated the hydroxynicotinic acid and the distilling apparatus at 110° for several hours before distilling. The receiver was also fitted with a $CaCl_2$ tube in order to exclude all traces of moisture. From a recent publication of Williams [1917] which reached us only lately we find that he independently adopted a similar procedure and like ourselves was able to obtain needles only. On cooling, the needles crystallise out from the syrup and finally complete solidification occurs with formation of a dense mass. In parts of the apparatus where the layer of crystals is thin, well-defined needles are discernible even after complete cooling down. The solidified syrup did not show a definite melting point, but melted somewhere between 94°–98°. On keeping the open apparatus under ordinary laboratory atmospheric conditions a marked change could be observed in the needles in those parts of the distilling flask where their crystalline form was well defined after complete cooling. The needles after about 10 days broke up by transverse fissures and ultimately changed into non-acicular crystals of a granular form. On heating the cooled down syrup at a temperature of 120° for 2–3 hours and then allowing the molten mass to cool slowly in the absence of moisture dissolving it in hot dehydrated benzene and adding dehydrated ligroin to the cold solution a milky turbidity was produced and after a short time a deposit of acicular crystals was formed. Few, if any, granules were obtained by this precipitation, but a preponderance of granular crystals was obtained on concentrating the mother liquor. The needles thus obtained melted at 108°–110° (uncorrected), whilst the granules melted at 106° 108° (uncorrected). It is to be remarked that the configuration of the needles obtained by the benzene-ligroin recrystallisation differed in the different preparations obtained by that method. We did not however study the conditions governing these differential configurations. When the freshly distilled α-hydroxypyridine is dissolved in hot water and crystallised from it a mixture of needles and prisms is obtained. It is thus seen that there are at least two distinct crystalline modifications of α-hydroxypyridine, namely needles and non-acicular crystals. It will however be remembered that the melting points of the needles obtained by direct distillation of hydroxynicotinic acid and the needles obtained by the benzene-ligroin crystallisation method are not the same and therefore the two varieties of needles cannot be considered to be identical.

Polyneuritis was induced in our pigeons by forcible feeding with rice, the birds receiving daily 20 g. each morning and evening. The crops of the birds were examined daily and if any hardness was observed the feeding was suspended until the bird became normal again. The time taken by the birds to develop polyneuritis varied from two to five weeks. All the injections were done intramuscularly in the breast and freshly prepared crops of crystals were always used unless otherwise mentioned. Following the practice of Williams the crystals were always dissolved in a convenient quantity of distilled water immediately before injection. The birds under treatment were kept under very close observation. Varying doses were tried. Needles obtained by direct distillation and needles from benzene and ligroin crystallisation as well as crystals obtained by crystallisation from hot water were tested. The following protocol describes eight experiments.

Expt. 1. 10 mg. of freshly distilled α-hydroxypyridine (needles) were dissolved in 2 cc. of distilled water and injected intramuscularly in pigeon 151 at 4 p.m. The bird showed at the time paralysis of the legs and retracted neck. At 8 p.m. its head was found to be markedly improved, and the condition of improvement was maintained at 11 p.m. Next morning the bird had a relapse. 20 mg. of needles obtained by distillation on the previous day were administered and as the bird showed no improvement a further 0·1 g. of the same substance was injected two hours later. The condition became worse and the bird died during the night.

Expt. 2. Pigeon 219 walked with great difficulty and was entirely unable to use its wings; head normal. It received 50 mg. of needles of freshly distilled α-hydroxypyridine at 12 noon. It was slightly worse at 11 p.m. the same evening, and worse again next morning. On the third day the bird showed a retracted neck. On the fourth day its condition was still worse and finally it was found dead on the morning of the fifth day.

Expt. 3. Pigeon 189 could not fly and walked with difficulty. It received 10 mg. of needles of freshly distilled α-hydroxypyridine at 12 noon. Next day there was no change in condition. On the third day the bird was decidedly worse. On the fourth day its condition was still worse, no retracted neck however was to be observed. It was found dead on the fifth morning.

Expt. 4. Pigeon 229 was paralysed and showed retracted neck. It received 50 mg. of needles obtained by recrystallising the α-hydroxypyridine from benzene with ligroin at 11.30 a.m. At 8 p.m. the same day its condition was worse and it died at 3 p.m. the following day.

Expt. 5. Pigeon 205 only showed incipient symptoms. It received 200 mg. of freshly distilled needles at 11.45 a.m. It became worse during the day and developed a retracted neck. Next day an improvement was observed but it became worse again in the course of the day and was found dead on the morning of the third day after treatment.

Expt. 6. Pigeon 230 showed paralysed limbs. It received 2 mg. of freshly distilled needles at 10 a.m. No change occurred in its condition during the day, but it became gradually but decidedly worse during the next three days and died on the fourth day.

Expt. 7. Pigeon 222 showed paralysed limbs. It received 50 mg. of α-hydroxypyridine recrystallised from hot water at 10.30 a.m. No change in condition occurred during that day. The next day the bird was worse and developed a retracted neck. It became gradually worse during the day and was found dead the next morning.

Expt. 8. Pigeon 149 showed paralysed limbs. It received 10 mg. of needles obtained by recrystallisation from benzene with ligroin at 6 p.m. No improvement was observed at 11 p.m. the same evening and the condition remained unchanged for some time. On the fourth day the bird was worse. 50 mg. of needles of the same crop were injected at 10.30 a.m. on that day. The bird died at 1 o'clock in the afternoon.

It is seen from the above experiments that no cure or improvement in the condition of the birds was effected by the treatment. Some birds lived several days after being treated, but they were types of cases, well known to those who work with polyneuritic birds, which would have behaved similarly if they had received no treatment at all. In many of the above experiments we started our treatment as soon as polyneuritic symptoms manifested themselves in order to give the drug a good chance to act. Some of these cases have turned out to be of the prolonged type where the onset is mild and gradual and lasts several days before terminating fatally, others have proved themselves to be of the intermittent type, in which the well-declared symptoms are strikingly relieved for a few hours before the second onset takes place; after the second attack the birds usually succumb. Expts. 2, 3, 6, and 8 are characteristic of the prolonged type. Expts. 1 and 5 represent the intermittent type. Special attention is to be paid to Expt. 5. That bird turned out to be of the intermittent type. It was treated when the symptoms were not more than of a premonitory character and the dose it received was very high, namely 200 mg. In spite of that, the first attack developed in the usual way and the bird succumbed as is common in cases of this type after the second onset within two days of the manifestation of the early symptoms. A special experiment showed that the injection of this amount of α-hydroxypyridine produced no toxic symptoms in a normal pigeon. In spite of the fact that we used both varieties of needles in various doses we are unable from our experiments to attribute any antineuritic properties to the needle variety of α-hydroxypyridine.

Reference was made above to the work of Williams and Seidell [1916] in connection with the isomerisation of adenine. They obtained an active substance from autolysed yeast by adsorption which they considered to be adenine and which on keeping became inactive. The inactive adenine on being treated with sodium ethylate in a sealed tube at 100° for four hours, according to the observations of these workers became active. While we were engaged in repeating some of these experiments there appeared a paper by Voegtlin and White [1916] in which a variety of experiments with adenine, all of a negative nature, were described. We have therefore repeated only the crucial experiments of Williams and Seidell, and like Voegtlin and White were unable to confirm their results.

We prepared our adenine from a sample of pure adenine sulphate kindly supplied to us by Mr A. Chaston Chapman. The adenine sulphate which was recrystallised several times was obtained by Mr Chapman by hydrolysis from yeast nucleic acid. The adenine was precipitated from the sulphate by neutralising it exactly with ammonia and passing a stream of CO_2. The filtered residue was washed with water, alcohol and ether. The adenine gave Kossel's reaction; when heated quickly it melted at 343° (uncorrected) and yielded a picrate consisting of yellow silky needles which charred at 240°. In accordance with Williams and Seidell's directions 50 mg. of adenine were heated at 100° for five hours with 5 cc. of absolute alcohol in which 10 mg. of metallic sodium were dissolved. The orange coloured liquid was then evaporated to dryness on a water-bath and the residue dissolved in distilled water immediately before injection. The following protocol describes five experiments.

Expt. 9. Pigeon 256, which showed well-developed symptoms of polyneuritis with retracted neck, received 10 mg. of untreated adenine; it became worse next day and was found dead on the morning of the third day.

Expt. 10. Pigeon 187 showed paralysed limbs. It received at 4 p.m. the entire residue from 50 mg. of adenine treated as described above. Slight improvement was noticeable on the next day when it again received the residue from 50 mg. of adenine. No change occurred on the third and fourth days: it became worse on the fifth day and remained in the same state during the sixth. On the seventh day it became decidedly worse and was treated with the residue from 50 mg. of adenine. No improvement was observed and the bird died during the night.

Expt. 11. Pigeon 247 showed well-declared symptoms of polyneuritis with retracted neck. It received at 4 p.m. 10 mg. from residue obtained by treating 50 mg. of adenine. The bird was found dead next morning.

Expt. 12. Pigeon 198, which was paralysed but kept its head well forward, received 5 mg. of treated adenine. On the next day it was a little better and received another 5 mg. of the adenine preparation of the previous day in the morning and further 5 mg. of a fresh preparation in the afternoon. On the third day the condition was unchanged and the remaining portion of the 50 mg. of adenine, treated on the previous day, was administered at 10.30 a.m. The bird became worse and died at 2 p.m. the same day.

Expt. 13. Pigeon 217 showed incipient signs of polyneuritis. It received 50 mg. of adenine treated with sodium ethylate. It was better the next day, but had a relapse on the third day and received a further 50 mg. of treated adenine. No improvement ensued and the bird was found dead on the fourth morning.

The above experiments like those performed with α-hydroxypyridine must be regarded as negative in character. In Expt. 10 the bird remained alive for six days, but the condition of the bird during that time was such that nothing could be definitely ascribed to the action of the administered adenine. The results obtained in the remaining experiments are so distinctly negative that no comment is necessary.

SUMMARY.

1. The authors can confirm the following facts observed by Williams about the nature of α-hydroxypyridine.

(*a*) α-Hydroxypyridine obtained by distilling hydroxynicotinic acid crystallises in needles and non-acicular crystals.

(*b*) On keeping the needles of α-hydroxypyridine, thus obtained, exposed under ordinary laboratory atmospheric conditions, they break up into granules in about 10–14 days.

(*c*) Needles of α-hydroxypyridine are also obtained by heating the freshly distilled substance for several hours at 120° and then adding ligroin to a solution of the cooled substance in benzene.

2. The authors find that the needles obtained from benzene-ligroin differ in their melting point from those obtained by direct distillation of α-hydroxypyridine.

3. Both varieties of needles of α-hydroxypyridine were injected into

polyneuritic pigeons but no cure or amelioration in the condition of the birds was observed. The observations of Williams were therefore not confirmed.

4. Crystals obtained by recrystallising α-hydroxypyridine from water proved also to be inactive.

5. Pure adenine, as well as adenine treated with sodium ethylate in a sealed tube for five hours at 100°, yielded negative results in agreement with Voegtlin and White but in disagreement with Williams and Seidell.

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ON THE OCCURRENCE OF HYDATID CYSTS IN MONKEYS.

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(*From the Lister Institute of Preventive Medicine.*)

In 1909, Professor McIntosh of St Andrews University sent me a cyst from the abdominal cavity of a South African baboon, *Cynocephalus porcarius* Budd. The cyst, which was single, had been ruptured in removal, part of its contents had escaped, and some blood had entered it. The cyst measured about 20 mm. in diameter, was a typical hydatid, and resembled in its general structure the ordinary simple unilocular hydatid of man.

Attached to the cyst wall and scattered throughout the grumous contents were numerous scolices. These were ovo-globular in outline and measured on an average 0·215 × 0·175 mm. The head or body of the scolex measured 0·095 × 0·12 mm. while the tail measured 0·12 × 0·055 mm. The four suckers measured approximately 0·054 mm. in diameter but the size varied from 0·042 mm. to 0·063 mm.

The head was surmounted by a double row of hooks, rather straight in type, and measuring 0·021–0·027 mm. in length. The average number of hooks was 38.

As is well known the hydatid tapeworm which passes its adult stage in carnivores, particularly dogs, is one of the most widely distributed parasitic worms known. It occurs in greatest profusion in countries and districts where dogs or other *Canidae* are numerous and especially where there are many cattle and sheep. Few parasites, however, possess such an extensive and varied list of intermediate hosts and the number of species of animals known to function as such probably exceeds forty.

Man, unfortunately, is by no means an uncommon intermediate host and is only too frequently fatally affected. Short of death the parasite gives rise to many symptoms of the most serious nature, the only remedy for which in most cases is operative treatment.

Amongst the numerous animals which figure as intermediate hosts mention may be made of cattle, sheep, pigs, deer of various kinds, goats, horses, donkeys, dogs, rabbits, kangaroos, etc. Several species of monkeys and apes are also already known as hosts of hydatids, for instance, *Inuus cynomolgus*, *Inuus ecaudatus*, and *Theropithecus silenus*. The camel, dromedary, giraffe and tapir also figure amongst the intermediate hosts. It would in fact appear as if all the larger Mammalia are possible hosts. It would indeed be of considerable interest to discover any species of the larger mammals which can be shown conclusively to be immune to infection with this parasite.

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